A Simple Method to Demonstrate Urate Crystals in Formalin Fixed Tissue

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Abstract: In clinically suspected cases of gout the surgical tissue specimens should be fixed in absolute ethyl alcohol, because urate crystals are soluble in aqueous formaldehyde.

In clinically not recognized cases of gout surgical specimens are usually fixed in formalin in which urate crystals may dissolve.

The aim of this study was to demonstrate the advantages of examining not staining sections under polarized light in comparison with haematoxylin-eosin stained ones viewed with normal light.

One hundred twenty five (125) tissue samples of 30 patients with gout were studied in serial sections stained with haematoxylin-eosin (examined by usual light microscope), or were studied in unstained sections (examined under polarized light) with an Olympus BX51 polarization microscope.

Urate crystals were demonstrable in 23.3% of formalin fixed tissue specimens of the patients stained with haematoxylin-eosin but were present in 56.7% of not stained tissue section viewed under polarized light.

Urate crystals were demonstrable in 43.7% of formalin fixed tissue specimens which were considered negative with haematoxylin-eosin stain.

The authors concluded that in case of gout the tissue specimens are best evaluated in sections stained with haematoxylin-eosin and in unstained sections as well. The probability of urate positive microscopic findings is significantly higher (χ^2=4.8700, p<0.027) in unstained sections viewed under polarized light than in the haematoxylin-eosin stained ones. This approach may also be useful in other crystal deposition induced diseases.

Keywords: Gout, urate crystals, methods of not-staining, polarizing microscope.

INTRODUCTION

The demonstration of urate crystals in tissue sections is an important, but not absolute diagnostic criterion of gout. The histochemical textbooks of Pearse (1985) [1], Lillie (1954) [2] or Vacca (1985) [3] present several method to demonstrate urates. One of the classical methods is Gömöri's methenamine silver method for urates (quoted by Pearse AGE, 1985; or Carson FL, 1990a) [4, 5]. Neither Pearse, nor others mention the simplest technic for the demonstration of urates in tissue sections, namely "not-staining".

In previous studies we found that urate crystals very often remain demonstrable in unstained sections in contrast to haematoxylin-eosin stained sections (Bély and Krutsay, 2013a,) [6]. Urate crystals are partly dissolved during fixation in aqueous formaldehyde and during dehydration by acetone before embedding in paraffin. However, the main reason of their dissolution is the haematoxylin staining of nuclei (Bély and Krutsay, 2013b) [7].

The aim of this study was to ascertain that a simple and most effective method for the microscopic demonstration of urates is "not-staining". We call attention to the importance of studying unstained tissue sections which are traditionally mounted with Canada balsam and cover slipped to be viewed under polarized light.

MATERIAL AND METHODS

One hundred twenty five (125) tissue samples of 30 patients (2 females. average age of 64.5 years, range 62 – 67; 28 males, average age of 52.4 years, range 76 – 35) with gout were studied in serial sections stained with haematoxylin-eosin and in unstained sections and examined under polarized light with an Olympus BX51 polarization microscope

The tissue blocks were fixed in 8% formaldehyde solution at pH 7.6 and embedded in paraffin. Serial sections were cut and stained with haematoxylin-eosin (Carson FL, 1990b) [8].

Unstained tissue sections were deparaffinized and traditionally mounted with Canada balsam and cover slipped.

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The efficiency of the "not-staining" method was calculated by χ^2-test, comparing the number of urate positive sections of the groups.

RESULTS

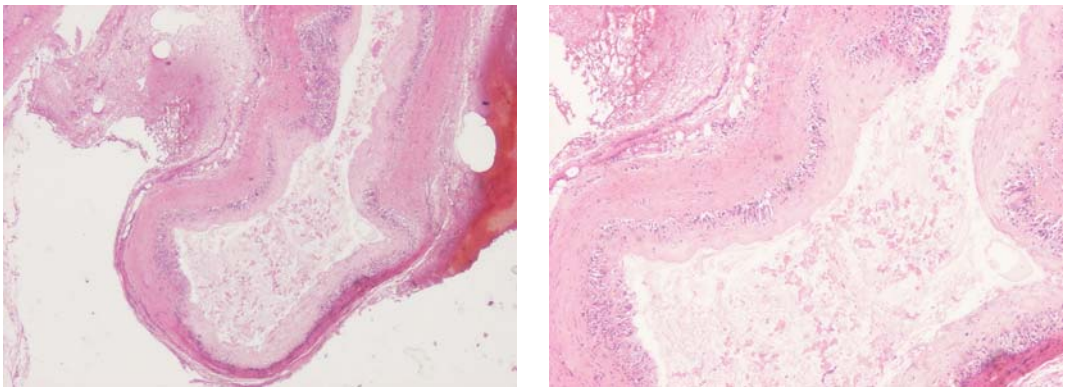
In formaldehyde fixed and haematoxylin-eosin stained sections, viewed under polarized light, urate crystals were detected in 7 of 30 patients (23.3%), whereas in unstained sections urate crystals were present in 17 of 30 patients (56.7%); the difference is significant. The probability of crystal positive cases was significantly higher (χ^2=4.87, p<0.03) in unstained sections viewed under polarized light in comparison with hematoxylin-eosin stained ones.

In 43.7% of "urate negative" cases, stained with haematoxylin-eosin (in 10 of 23 patients) (Figures **1a-b** and **2a-b**), urate crystals were demonstrable in unstained tissue sections (Figures **3a-d** and **4a-d**).

DISCUSSION

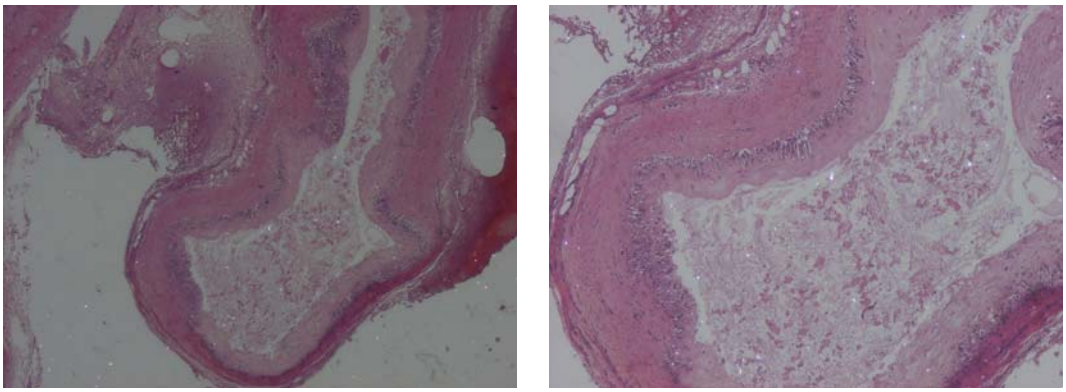
In clinically known or suspected cases of gout the surgical tissue specimens should be fixed in absolute ethyl alcohol, because urate crystals are soluble in 8% formaldehyde water solution. This statement summarizes the view of current textbooks (Lillie, 1954; Pearse, 1985; Carson, 1990; McManus and Mowry, 1960) [9]. According to Lillie (1954) "sodium urate …crystals are slightly soluble in cold water, and insoluble in alcohol and ether". The histotechnical handbook of McManus and Mowry (1960) concludes that "since urates are slightly soluble in water, alcohol fixation is preferable".

In some cases urate crystals remain demonstrable in formaldehyde fixed tissue specimens, stained with haematoxylin-eosin, and viewed under polarized light. In these cases the presence of urates is explained by large amounts of crystals in tissue specimens.



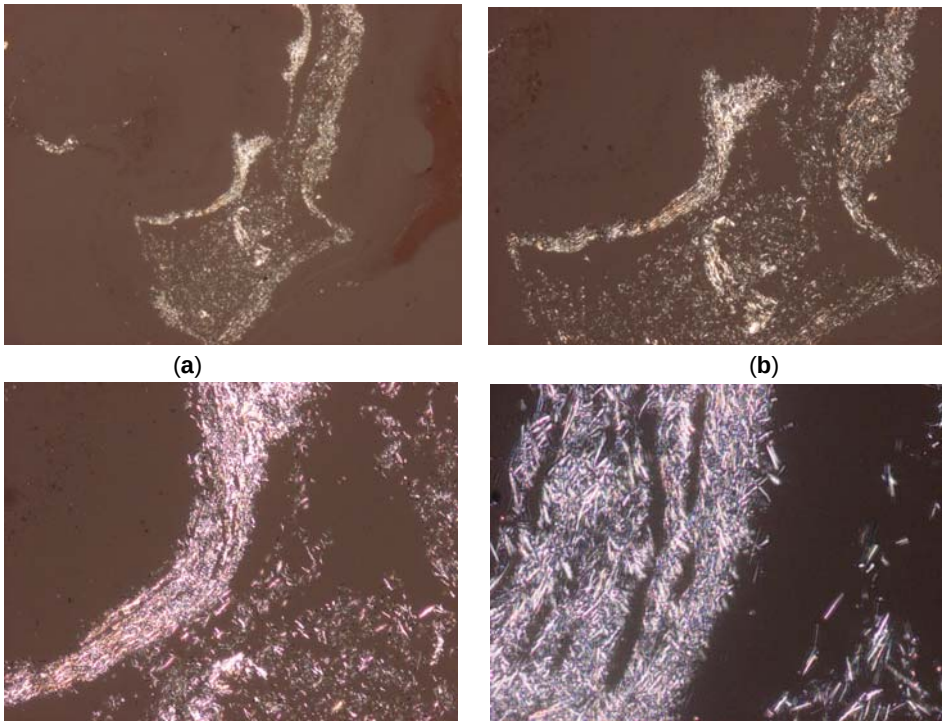
(**a**) (**b**)

Figure 1: Tophus uraticus, under the light microscope.
(**a**) HE, x 20, (**b**) same as (**a**) x40.



(**a**) (**b**)

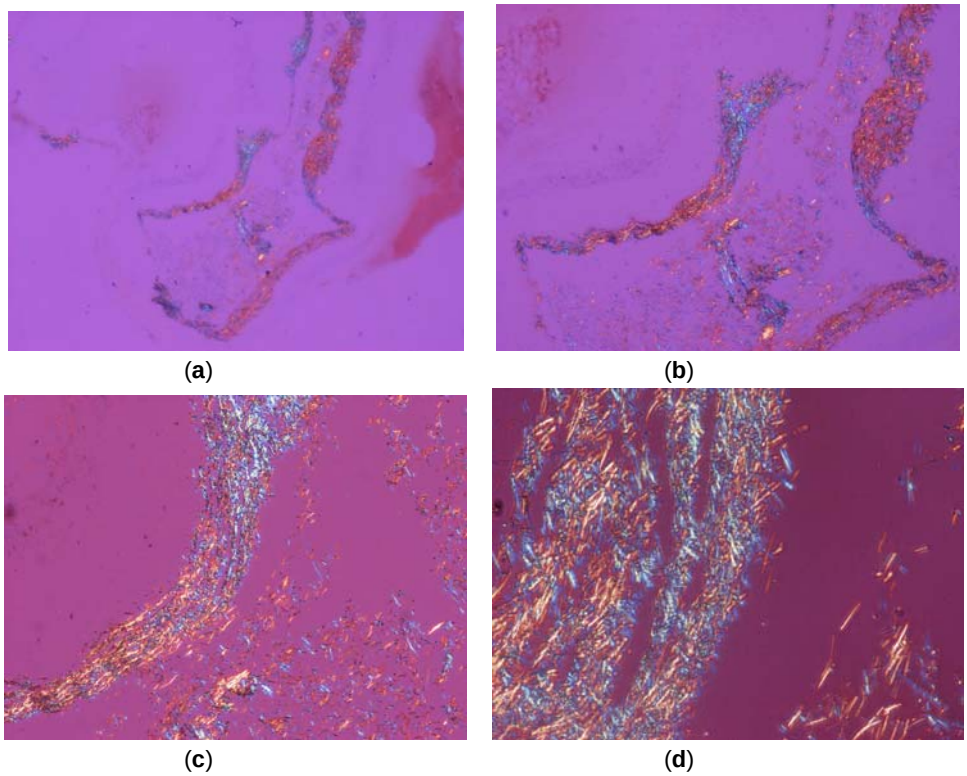
Figure 2: Tophus uraticus, viewed under polarized light (same as Figure **1a-b**).
(**a**) HE, x 20, (**b**) same as (**a**) x40.
Urate crystals are not present in 8% formaldehyde fixed specimens, haematoxylin-eosin stained sections.



(**a**) (**b**)

(**c**) (**d**)

Figure 3: Tophus uraticus, unstained sections, mounted with Canada balsam, cover slipped, and viewed under polarized light (same as Figure **1a-b**).
(**a**) x20, (**b**) same as (**a**) x40, (**c**) same as (**a**) x100, (**d**) same as (**a**) x400.
Urate crystals are present in 8% formaldehyde fixed specimens in unstained sections.



(**a**) (**b**)

(**c**) (**d**)

Figure 4: Tophus uraticus, unstained (native) sections, mounted with Canada balsam and cover slipped, Rot I. compensator, and viewed under polarized light (same as Figure **1a-b**).
(**a**) x20, (**b**) same as (**a**) x40, (**c**) same as (**a**) x100, (**d**) same as (**a**) x400.
Urate crystals are present in 8% formaldehyde water solution fixed specimens in unstained sections, showing a strong negative birefringence with Rot I. compensator.

Our results indicate that the simplest and most effective technique to demonstrate urates is "not-staining" (Bély and Krutsay, 2013a). We suggest analyzing tissue specimens in case of suspected gout, independent of alcoholic or formaldehyde fixation, in unstained tissue sections as well, and to be examined under polarized light. Urate crystals remain detectable in unstained sections in the great majority of "urate negative" sections stained with haematoxilin-eosin (Bély and Krutsay, 2013a, 2013b) [6, 7].

In unstained sections urate crystals may remain sporadically even in sections of decalcified bone tissues, in spite of acidic decalcification (Bély and Krutsay, 2013a) [6].

Alcoholic fixation is very important to preserve the urates in tissues. Although the forefathers were right regarding the solubility of urates in aqueous solutions (Lillie, 1954; McManus and Mowry, 1960), the main reason of the loss of urates is the dehydration by acetone (before embedding in paraffin), and more over the most important is the staining of nuclei in haematoxylin (Bély and Krutsay, 2013b) [7].

CONCLUSION

In case of gout the tissue specimens should be evaluated in sections stained by haematoxylin-eosin and in unstained sections as well. The probability of urate positive cases is significantly higher in unstained sections viewed under polarized light in comparison with the haematoxylin-eosin stained ones. This approach may also be useful in other crystal deposition induced diseases.

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