Technical methods

New methods applied to the identification of glycogen in pathological material

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Glycogen accumulates in large amounts in many abnormal conditions. The cytological demonstration of glycogen is routinely carried out using the PAS method or Best's carmine technique. Both these procedures have certain disadvantages, the former not being specific for glycogen and the latter not always producing reliable results. Both these methods require at least two sections, one being stained normally and the other (control) predigested with enzyme and then stained. The substitution of haematoxylin and alizarin dyes for carmine in the staining of normal glycogen has been recently reported (Murgatroyd and Horobin, 1969) and these methods have since been used successfully for the identification of glycogen in pathological material. A control procedure which involves the use of one slide only has been successfully applied.

Method

Small pieces of liver and kidney were taken from cases of glycogen storage disease, renal carcinoma, and diabetes mellitus. The tissues were fixed in neutral formalin and cold formol alcohol, processed through paraffin wax, sectioned at 5μ, and stained with haematoxylin, alizarin red S, and alizarin brilliant blue BS.

PREPARATION OF THE STAIN

One gram of haematoxylin, or alizarin red S, or alizarin brilliant blue BS, 1 g of potassium carbonate, and 5 g of potassium chloride are dissolved in 60 ml of boiling, distilled water. The solution is then cooled and 20 ml of the stain is added to 15 ml of concentrated ammonia (0·880 s.g.) and 15 ml of methanol then added. This staining solution should be prepared freshly for each batch of slides to be stained.

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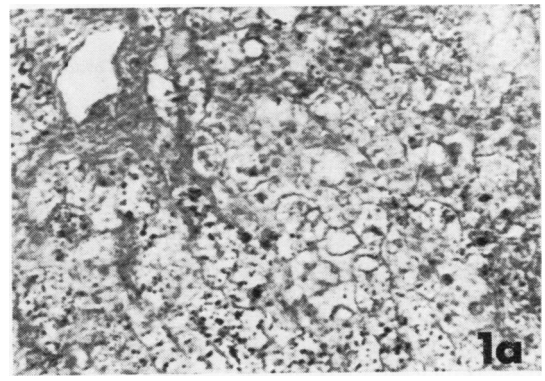
STAINING PROCEDURE

1 The sections are brought to water.
2 Sections are stained for about two to five minutes in the staining solution, preferably in a Coplin jar.
3 The slides are then rinsed in pure methanol to wash off excess stain.
4 Counterstaining if required is then carried out, followed by dehydration, clearing, and mounting in a suitable synthetic resin.



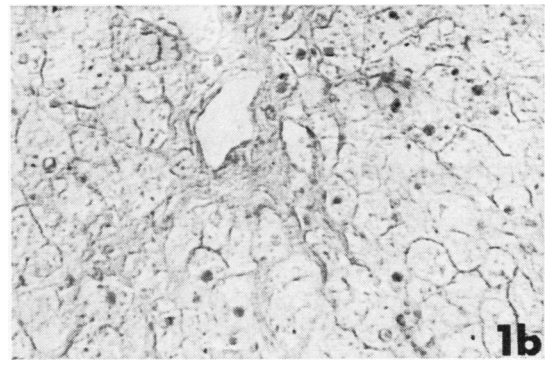


Fig. 1a *Section of human liver from a case of glycogen storage disease. This material was taken from an old block due to the rarity of the condition and fixation was in 10% formalin. Some of the glycogen will unfortunately have been lost. Stained with alizarin brilliant blue BS.* × *100.*
Fig. 1b *Same section as in Fig. 1a after enzymic digestion and restaining with alizarin brilliant blue BS. Note that all staining due to glycogen has been lost. Background staining which is present is due to the double staining procedure.* × *100.*

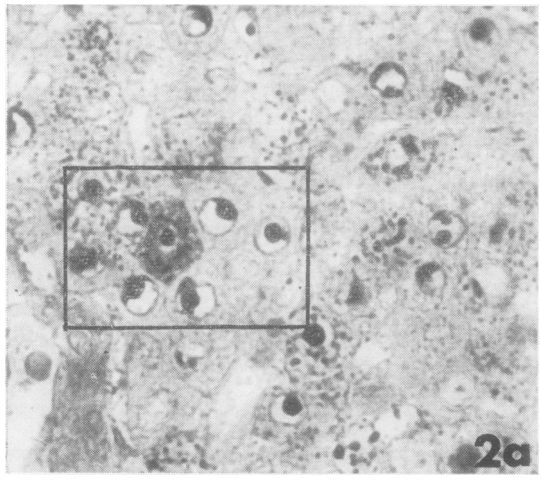


Fig. 2a *Section of human liver from a case of diabetes showing intranuclear glycogen. This material was fixed in chilled formol alcohol and stained with haematein.* × *400.*

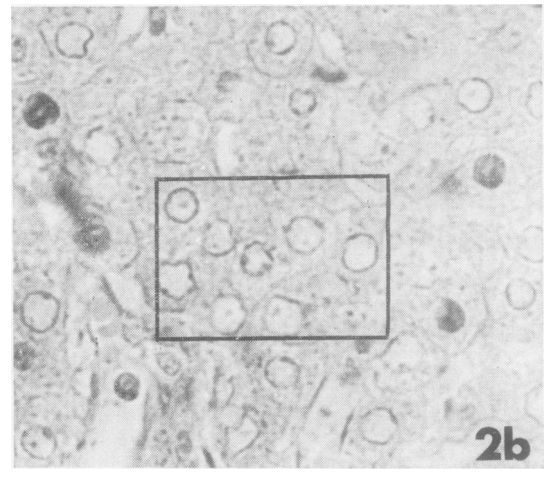


Fig. 2b *Same section as in Fig. 2a after enzymic digestion and restaining with haematein. Note loss of glycogen, particularly in marked area, allowing a direct cell-to-cell comparison to be made.* × *400.*

CONTROL PROCEDURE

Staining of glycogen was controlled using the method of Goldstein and Murgatroyd (1968). The sections were stained and photographed, then digested with saliva, diastase or diazyme and restained for glycogen by the original procedure. The same area was then photographed again, thereby obtaining a direct comparison before and after digestion (Figs. 1a and 1b and 2a and 2b).

COUNTERSTAINING

The technique worked better if counterstaining was omitted but where required, it was carried out in the following manner. When haematein or alizarin red S was used, counterstaining with Ehrlich's haemalum after stage 1 in the staining procedure proved acceptable. When alizarin brilliant blue BS was used, counterstaining with a 1% alcoholic/alkaline 50:50 solution of safranin after stage 3 produced good results. It is not possible to counterstain before staining the glycogen with this latter method.

Comment

Glycogen is stained dark red with haematein or haematoxylin which, due to further oxidation, gradually turns dark brown. The colour at this stage seems stable. Glycogen is stained red with alizarin red S and deep blue when alizarin brilliant blue BS is used.

These staining methods are recommended for routine use since they offer technical advantages over existing methods. No differentiation is required and the staining time is rapid. Precipitation does not occur if staining is carried out in a closed container and the methods are less complex than the PAS method. Histochemically, these methods have the advantage that background and nuclear staining are less than with Best's carmine or PAS.

The use of one slide only is, if photography is available, particularly helpful where intranuclear glycogen is present, since direct cell-to-cell comparisons can be made before and after the loss of glycogen (Figs. 2a and 2b).

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References

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Murgatroyd, L. B., and Horobin, R. W. (1969). Specific staining of glycogen with haematoxylin and certain anthraquinone dyes. *Stain Technol.*, **44**, 59-62.