Acetylated Sudan Black B as a More Specific Histochemical Reagent for Lipides

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SUMMARY

Sudan black B is treated with an equivalent of acetic anhydride. The acetylated product is more specific for lipides than the parent dye.

ACETYLATED Sudan black B appears promising as a more specific reagent for the histochemical demonstration of lipides. This and other derivatives of Sudan black B, Sudan IV, and oil red O were introduced by Lillie and Burtner in 1953. Although the formulae for Sudan black B given by these authors and by Conn (1953) differ, they do agree on the presence of two secondary amino-groups. These are, undoubtedly, the sites of acetylation.

Lillie and Burtner (1953) treated Sudan black B with a large excess of acetic anhydride in pyridine. With some brands of the dye, appreciable decomposition occurs, yielding orange or brown substances which are not lipide colorants. This seldom occurs, however, if Kaufmann's (1909) method of acetylation is used with an equivalent of acetic anhydride in an inert solvent. The addition of sulphuric acid as a catalyst (Fieser and Martin, 1935) is unnecessary and, sometimes, appears to have resulted in an inferior product. The following method is suggested:

Dissolve 1 gm. of Sudan black B in 100 ml. of diethyl ether. Filter the solution to remove a lipide-insoluble fraction. Under a reflux condenser, heat the etherial solution to boiling and add 0·5 ml. of acetic anhydride in 20 ml. of ether. Reflux for 20 minutes. Cool and filter the mixture. Transfer the filtrate to a separating funnel. Extract it repeatedly with cold water until the aqueous layer is no longer coloured and is not appreciably acidic ('universal' indicator paper). Pour the solution of acetylated Sudan black B into a dish and evaporate off the ether.

The black product has a metallic lustre. Like Sudan black B, it is stable in propylene glycol and moderately stable in 70 per cent. ethanol. In paper chromatograms, its principal fraction is dark blue-black. There are small amounts of bluish-grey components and, occasionally, a trace of a brownish one.

Acetylated Sudan black B may be used in the procedures described by Baker (1944), Lillie (1944), or Chiffelle and Putt (1951). Prepared as described above, it has been applied to a variety of histochemical analyses (Casselman, 1954*a*, unpublished; Shafiq and Casselman, 1954). From all sites of coloration, the acetylated derivative has been readily extractable with 70 per cent.

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ethanol; whereas, with Sudan black B, 'stable sudanophilia' has been observed for some structures, especially the 'lipochondria' in locust motor neurones (Shafiq and Casselman, 1954). In these cytoplasmic inclusions at least, the retention of the dye appears to be associated with a high content of phospholipides. Thus, the 'stable sudanophilia' first observed by Lillie and Burtner (1953) in the granules of leucocytes can, in some cases, be due to certain classes of lipides. In other instances, however, it is probable that this is due to some non-lipide constituent with which the Sudan black B might react as a basic dye. Because of this possibility, it is suggested that acetylated Sudan black B should be used in place of Sudan black B during histochemical analyses of lipides, especially where 'stable sudanophilia' is demonstrable with the parent dye.

The greater specificity of acetylated Sudan black B for lipides has also proven valuable in certain histological studies and in paper chromatography (Casselman, 1954*b*). In the case of the former, lipide-containing structures are still intensely coloured but there is little or no non-specific 'background' staining. This observation has been confirmed by Drs. C. L. Foster and R. R. Wilson who have kindly tested some of the preparations of acetylated Sudan black B.

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