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Analysis of Orcein Dye for Hepatitis B Surface Antigen Staining by Using Thin Layer Chromatography and Mechanism of Non-specific Reactions

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Abstract: About ten analogs comprising orcein dye have been separated using thin layer chromatography. The orcein dye spotted on reverse phase thin layer chromatography plates were developed with a solution of 100% acetone 9 parts, 28% ammonium hydroxide 1 part. The orcein dye has different staining properties from lot to lot, causing difficulty in obtaining stable and satisfactory results. As the results, different orcein analogs sometimes give different results. If the orcein dyes contain negatively charged dye, protein reacts with it because of coupling of the electric charge. Therefore, it is suggested that the orcein dye should be selected.

Key words: Hepatitis B surface antigen, Orcein, Thin layer chromatography.

INTRODUCTION

Many methods have been developed for the purpose of staining hepatitis B surface antigen in paraffin sections, including an orcein method (Shikata *et al.*, 1974), modified orcein method (Senba, 1982, 1983), aldehyde fuchsin method (Shikata *et al.*, 1974), aldehyde thionine method (Shikata *et al.*, 1974), Victoria blue method (Tanaka *et al.*, 1981), and resorcin fuchsin method (Senba, 1982). Among these Shikata's orcein method was the first one that used dyes, and has been widely employed. This method was compared with the immunofluorescent method (Shikata *et al.*, 1974, Tanaka *et al.*, 1981) and the material stained with orcein and Victoria blue was suggested to be hepatitis B

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surface antigen. The orcein dye varies considerably in staining properties by brand and lot number, resulting in difficulty in constantly obtaining stable results. Therefore, we attempted to separate the orcein dye using thin layer chromatography.

MATERIAL AND METHOD

Thin layer chromatography No. 5554, TLC aluminium-sheet silica gel 60 F254 was used for orcein dye analysis.

The solvent system which gave the best separation consisted of 100% acetone 9 parts, 28% ammonium hydroxide 1 part.

An applicator was used to spot the plates about 1.5 cm from the bottom. Each sample was dried with a hair dryer immediately after spotting.

RESULTS

The orcein analogs (Fig. 1) were separated various colors brown, violet, blue and orange on the thin layer chromatography. The appearance of a developed plate is shown in Figure 2.

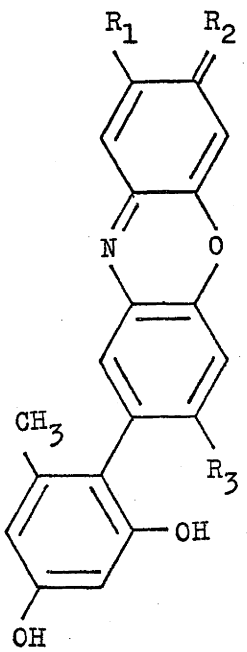

Fig. 1. Chemical structure of orcein dye (From the references No. 6: The Merck Index)

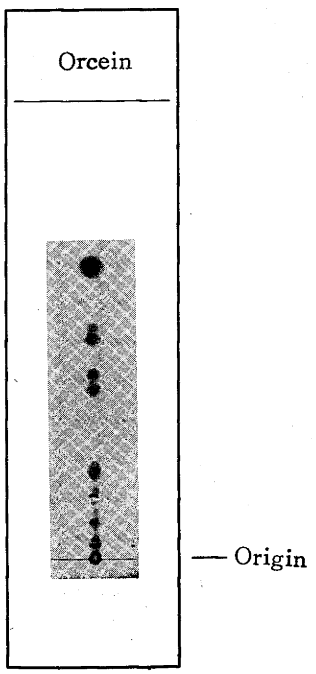

Fig. 2. Developed thin layer chromatography plate showing orcein analogs separated.

DISCUSSION

The orcein dye reacted not only with sulfonic acid residue groups, but also with small gaps in the protein molecular structure in the cell. Therefore, a more satisfactory method of staining hepatitis B surface antigen by two metal salts in histologic sections was introduced (Senba, 1982). However, rare nonspecific reactions did occur in histologic sections. As shown in Figure 3, in the reaction in which amino groups react with hydrogen in an acidic solution ($^{+}NH_3$-R-COOH) the electric chage of proteins becomes positive as a result (Senba, 1982, 1983). The orcein can be subdivided into 14 dyes by distribution chromatography (Windholz, 1976). If the orcein dyes contain negatively charged dye, protein reacts with it because of coupling of the electric charge. Therefore, different orcein analogs sometimes give different results.

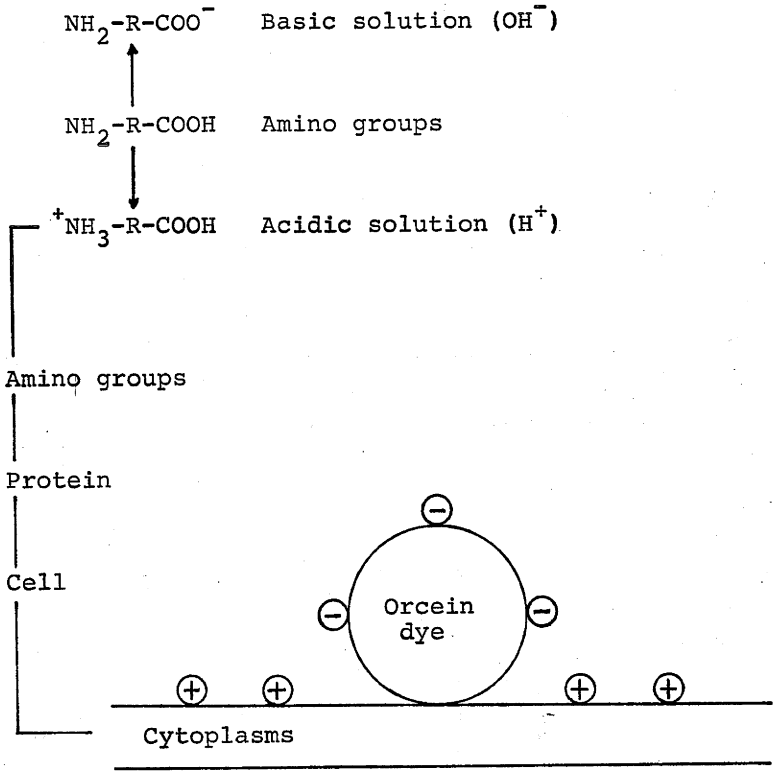

Fig. 3. Non-specific reaction of orcein dye

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薄層クロマトグラフィーによるオルセイン色素の分析およびB型肝炎ウィルス染色に於る非特異的反応について

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B型肝炎ウィルス表層抗原染色において，オルセイン色素を一般的に使用する．オルセイン色素はメーカーやロット番号で染色性にかなり差があり，常時安定した結果を得ることがむずかしい．この理由はオルセイン色素が14種類の組成よりなるためである．我々は薄層クロマトグラフィーを用いてオルセイン色素の分析を試みた．展開溶媒は100%アセトンと28%アンモニア水を9：1の割合で行った．この結果，純粋な単一成分ではなく約10種類の組成からなることが解った．また，B型肝炎ウィルス表層抗原染色に於る非特異的反応については，負に荷電するオルセイン色素によるのではないかと考察した．

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