Rhodamine B Fluorescence as a Stain for Amniotic Fluid Squames in Maternal Pulmonary Embolism and Fetal Lungs

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ABSTRACT

Rhodamine B fluorescence is presented as a simple, rapid, highly sensitive, permanent method for the histologic detection of squames in maternal amniotic fluid emboli and fetal lungs in cases of intrauterine asphyxia. The method may be used on alcohol fixed smears or formalin fixed paraffin sections. The application of this procedure allows for identification of sparsely distributed fetal squames which otherwise may be overlooked by less sensitive tinctorial methods which may also be more tedious in technical preparation and screening.

Introduction

Amniotic fluid containing squamous cells shed from the surface of fetal skin (vernix caseosa) can be found as emboli in the maternal circulation or aspirated into the fetal lung. Although the pathogenesis of amniotic fluid embolism is not well established, it has been considered that a sudden and sometimes massive obstruction of the pulmonary vasculature may occur in late pregnancy, during labor, or shortly after delivery, both in spontaneous delivery as well as during Caesarian section. This may lead to shock or in some cases even death secondary to hypoxemia, circulatory collapse, or coagulation defects. Left ventricular failure is the only hemodynamic abnormality consistently observed in humans. This may be secondary to the entrance of amniotic fluid into the maternal circulation.

The characteristic histologic pattern of lungs of a mature fetus may appear altered by intrauterine anoxia, external compression, or malfunction. If anoxia stimulates increased respiratory activity, more amniotic fluid may be aspirated into potential air spaces. Concomitantly with increased fetal maturity, more desquamated epithelial cells and lanugo hairs are present in the amniotic fluid and can be aspirated. Squames can be found in the lungs of stillborn fetuses, particularly mature ones, and those expiring shortly after delivery. Intrauterine asphyxia, a critical consequence of obstruction of the placental circulation, initiates premature fetal respiratory movements and consequent aspiration of

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amniotic fluid with vernix caseosa and cornified epithelial cells contained therein. The identification of cornified squames is essential as evidence of this event.\textsuperscript{1,2,7,12,14,15,16}

A technique utilizing a mixed stain of phloxin with alcian green has been reported to demonstrate fetal squames.\textsuperscript{1} Routine hematoxylin and eosin, a modified Mallory trichrome,\textsuperscript{4} and a keratin mucin procedure have been used in this laboratory to identify maternal pulmonary amniotic fluid embolism. Rhodamine B fluorescence has been found to be a selective stain for keratin-like proteins in skin stratum corneum\textsuperscript{6} and in Mallory bodies.\textsuperscript{18} The present report evaluates rhodamine B fluorescence as a means of identifying amniotic sac squames in maternal blood and fetal lungs.

Materials and Methods

Two cases of women showing amniotic fluid embolism in venous blood smears were studied. Four cases of fetuses or stillborns with amniotic fluid aspiration in lungs were used. Ten cases of fetuses or full-term stillborns uninvolved with amniotic fluid aspirations, normal human skin, and six cases of squamous cell carcinoma served as controls. Smears were made from maternal blood drawn from the right pulmonary artery on acid cleaned slides by a technician wearing surgical gloves to prevent introduction of exogenous squames. Smears of venous blood and formalin fixed tissue blocks of lungs were stained with hematoxylin and eosin, Kreyberg technique, and the rhodamine B procedure.

Fixation

Ten percent neutral buffered formalin is used for tissue, and absolute ethanol is used for smears.

1. Decorate three to six mu paraffin sections in three changes of xylene for five minutes each.
2. Hydrate through three changes of absolute alcohol; 95 percent alcohol; 70 percent alcohol; distilled water, at three minutes each. Smears are washed in running tap water for three minutes.
3. Stain in 0.1 percent for aqueous toluidine blue for 10 minutes.
4. Rinse three times in distilled water.
5. Stain 0.1 percent rhodamine B* in 0.1M McIlvaine’s buffer at pH 3.6 for 10 minutes.
6. Rinse three times in distilled water.
7. Dehydrate rapidly in ascending ethanol starting with 95 percent alcohol.
8. Clear in three changes of xylene.

Fluorescence microscopy was carried out using a Leitz Orthoplan microscope with a 200 W Osram HBO lamp and BG12 primary and K530 secondary filters.

Results

Venous blood samples from both cases presenting amniotic fluid embolism showed squames as scattered irregular, homogeneous eosinophilic, or red fragments between blood elements with hematoxylin and eosin and Kreyberg methods, respectively. Rhodamine B fluorescence demonstrated these moieties and lanugo as vivid bright yellow or yellow orange tissue fragments against a dark background (figure 1). Paraffin sections from four cases of full term infant or fetal lungs, which contained material from the amniotic sac, showed some or many squames within alveoli. These were also identified in all cases with hematoxylin and eosin and Kreyberg stains. One case required extensive

\* Allied Chemical Company, National Anilino Division C.I., Sinking Spring, OH 54170.
screening to localize the squames after conventional stains. In contrast in all cases screened with rhodamine B fluorescence, the squames, even though they were sparse in distribution, were rapidly identified as needle-like or irregularly rounded bright yellow orange tissue fragments against the pale orange or green background of lung parenchyma (figure 2). Lungs from fetuses or full-term stillborns used as controls showed no squames. The stratum corneum of normal skin and all six cases of squamous cell carcinoma stained positively with rhodamine B fluorescence.

Discussion

Pregnant women at term or during delivery with clinical signs of pulmonary embolism show amniotic fluid containing particulate matter, such as fetal desquamated squamous cells, sebaceous material, and mucous, which can be identified in venous blood. Although a direct connection between amniotic fluid embolism and circulatory collapse has not been elucidated, the histologic detection of squames in maternal circulation and prompt therapeutic intervention is crucial. It is therefore essential to identify amniotic fluid embolism, which may occur in late pregnancy during labor or shortly after delivery, both in spontaneous delivery as well as in Caesarian sections.

Amniotic sac contents in fetal lungs stained with hematoxylin and eosin are not always readily evident in microscopic sections and may be occasionally over-
looked with conventional stains for keratin and acid mucopolysaccharides.

Rhodamine B has been introduced as a specific stain for cornification at the light microscopic level. The mechanism by which this stain binds keratin is not presently known. It has been reported that the intensity of this weakly basic xanthene derivative could be greatly enhanced by the use of fluorescent microscopy. In the present techniques, toluidine blue binds nucleic acid and mucopolysaccharide moieties, some of which also show affinity for the fluorochrome. In the past, rhodamine B has been reported to stain lipids, certain connective tissue elements, and mycobacteria. In this laboratory, the dye has been found to demonstrate Mallory bodies sensitively and selectively in murine and human liver.

Special stain procedures, such as Attwood’s stain, have been utilized in an attempt to facilitate identification of amniotic squames. An immunoperoxidase technique for human keratin was found to be sensitive and selective for their detection. However, the immunohistochemical technique is costly, tedious, and time consuming. Staining with rhodamine B and viewing with fluorescence microscopy is highly sensitive for the demonstration of squames and facilitates accurate determination of the true incidence of amniotic fluid emboli and their relationship to maternal death. The rhodamine B technique is reproducible, selective, permanent, and allows ready identification of bright orange-yellow squames against a pale or dark background with rapid fluorescent microscope scanning even when squames are scant.

References