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Fluorescence of nile blue sulphate in amniotic fluid cytology

by

G. D. MONTANARI, P. GRELLA and S. AGOSTI

INTRODUCTION

Amniotic fluid cells are primarily examined to diagnose rupture of the membranes and to evaluate foetal maturity. Nile blue staining is helpful for both these purpose, although with some disadvantages. Orange-stained cell counts are influenced by clumping of the cells and by consequent difficulty of differentiating large drops of extracellular fat from foetal squamae. Interpretation may also be impeded by stain or crystal deposits, or by the presence of maternal squamae. The increase of lipid cells in the later stages of pregnancy has also been differently reported in the literature.

Earlier work ([3]) showed Nile blue sulphate fluorochroming for cell components and extracellular material stained orange in ordinary light microscopy; these are probably neutral fats. The present paper reports results observed in an investigation designed to show whether this feature of the dye is of assistance in increasing the accuracy of the methods proposed by Kittrich ([2]) and Brosens & Gordon ([1]) for the determination of foetal maturity and membrane rupture respectively.

MATERIALS AND METHODS

Foetal maturity was assessed from amniotic fluid sediments obtained during caesarian section (31 patients with unruptured membranes), by transabdominal amniocentesis in 18 cases of Rhesus incompatibility, and as a result of vaginal amniorrhexis via the amnioscope (38 cases). Contamination with cervical and vaginal material was virtually nil when the last method was amployed. To assesment of maturity were made:

a) by assessing the percent of orange-stained cells presentig counts of 300 cells under the ordinary light microscope;

b) by grading (from 0 to 4) the number of isolated or masses lipid cells and drops of extracellular fat observed in fluorescence microscopy.

The failure to observe non-lipid material, of course, prevents a differential

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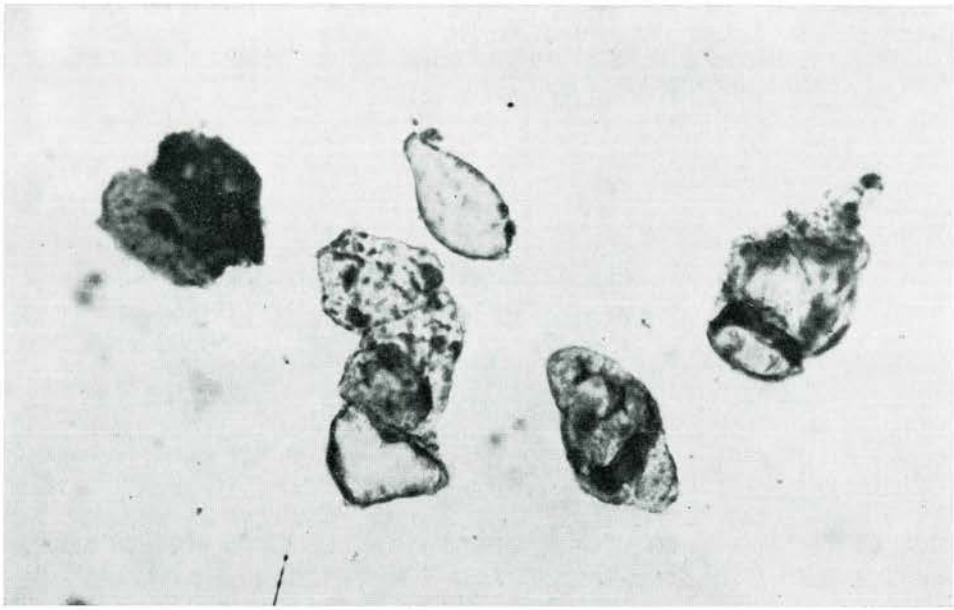

FIG. 1 - Foetal lipid cells. Nile blue sulphate. (Light microscopy x 250).

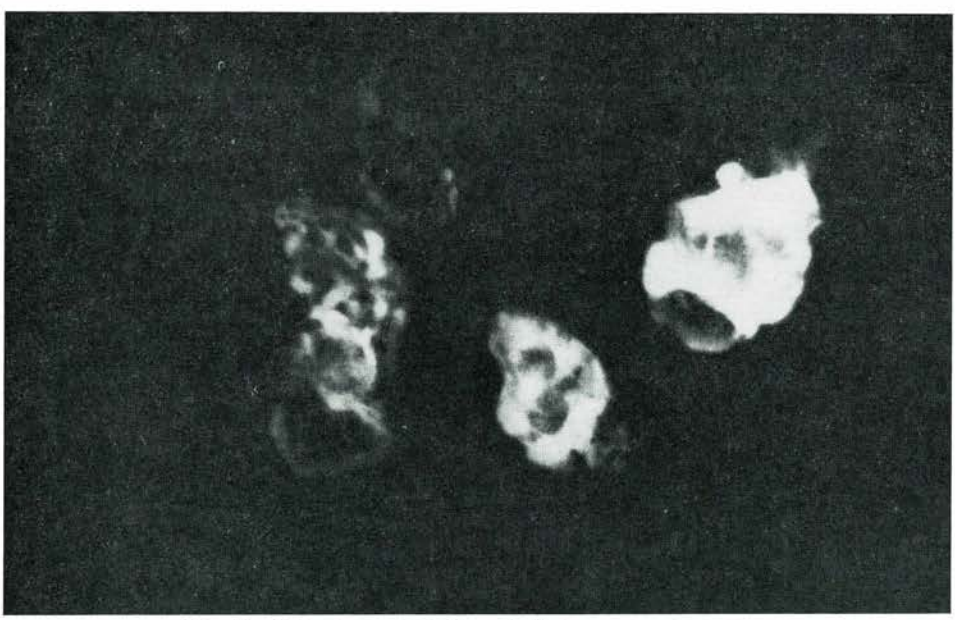

FIG. 2 - The same preparation at fluorescence microscopy.

count or at any rate an exact quantitative evaluation from being made by the latter method.

Membrane rupture as diagnosed from material withdrawn from the posterior fornix with a glass pipette fitted with a rubber bulb. 42 patients with certainly unimpaired and 38 with certainly rupturd membranes (all at term) were used for this purpose.

A drop of material, liquor amnior vaginal content, was placed on a slide and carefully mixed with a drop of 0,1 per cent Nile blue sulphate solution. After a delay of a few minutes the slide was covered and examined:

1) in ultraviolet light (excitation filter BG 12,5 mm. trick; blue absorbent blocking filter K 530); and, by the instantaneous shifting of a mirror;

2) in ordinary light.

As can seen in fig. 1, foetal squamae (lipid cells) appear orange in ordinary light, whereas their fluorescence colour is very bright yellow-green (Fig. 2). Other cells are more or less deeply stained blue in ordinary light and display no sign of fluorescence.

RESULTS

Foetal maturity: The results in 87 patients are plotted in the graph in Figg. 3 and 4. There is evidence that fluorescence microscopy may integrate the assessment with ordinary light, since it gives a good demostration of the gradual increase of foetal squamae masses and extracellular lipid drops that place as full term approaches.

Membrane rupture:

a) light microscopy gave no false negatives (0/38 cases) and 2/42 (5.2 per cent) false positives;

b) fluorescence microscopy gave 1/38 (2.6 per cent) false negative and 0/42 false positives.

It can be assumed that the two techniques are of similar reliability.

COMMENTARY AND CONCLUSIONS

One of the advantages of fluorescence microscopy in the examination of 0,01% Nile bue sulphate-stained amniotic fluid or vaginal content specimens is that it suppresses the non-lipid background. Complementing the data obtained under the light microscope may be helpful to solve doubtfull cases, especially since it enables cell counts to be made in relatively small lipid masses and can distinguish cells from extracellular lipid drops; this is of importance towards the end of pregnancy, when both drops and large cell masses increase in number.

The fact that non more than a qualitative evaluation may be obtained need not be regarded as a disadvantage, since the distinction between isolated cells, cell masses and extracellular drops is often so clear that non more than a glance is required to make an assessment to foetal maturity. The fact that the equipment is expensive is, of course, a point in its disfavour.

By comparison with acridine orange, Nile Blue sulphate gives a much clearer picture. Squamal fluorescence is more pronounced, while the acridine orange dye is unable to suppress the background. The 3:4-benzopyrene has been sug-

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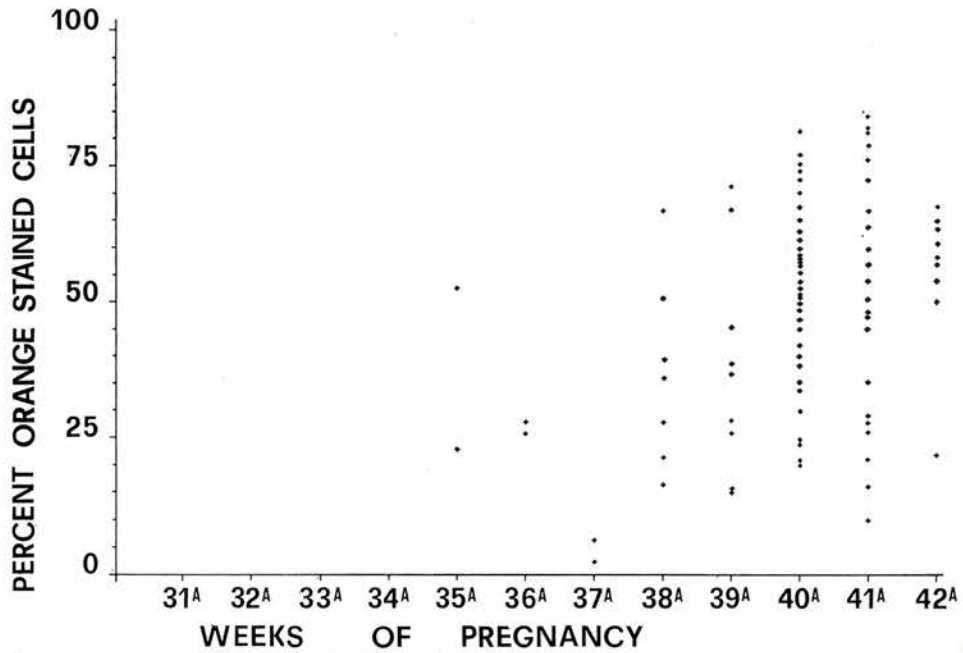

FIG. 3 - Per cento orange-stained cells in relation to pregnancy period. Nile blue sulphate (Light microscopy).

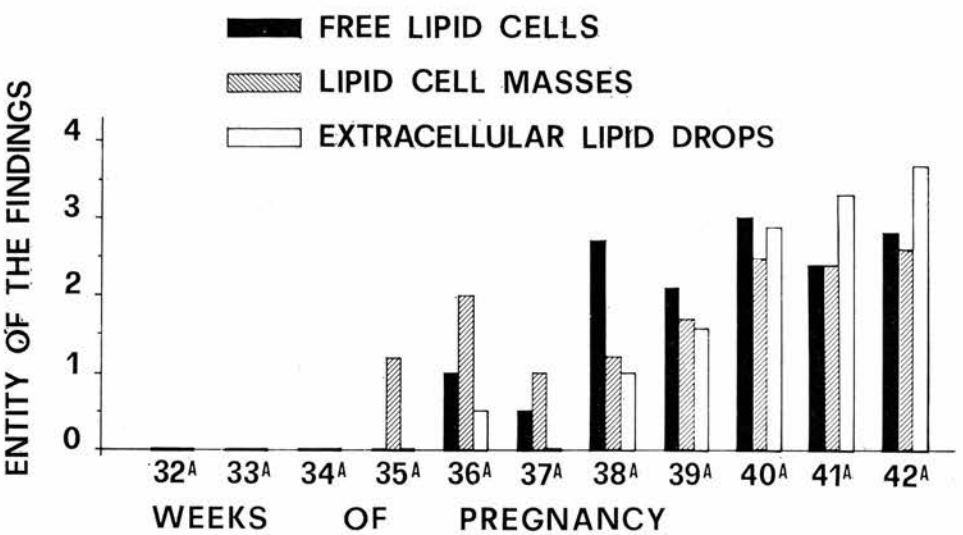

FIG. 4 - Amniotic fluid sediment picture in relation to pregnancy period. Nile blue sulphate (Fluorescence microscopy).

gested as a lipid- specific fluorochroming substance in the diagnosis of rupture of the membrane ([4]). Nile blue sulphate, however, has the advantage that is free of danger for the user.

SUMMARY

Results obtained with fluorescence microscopy in the diagnosis of rupture of the membranes and in assessment of foetal maturity Nile blue sulphate are presented.

Foetal lipid cells, stained orange when viewed in ordinary light, display a deep yellow-green fluorescence and the method thus enables an immediate double evaluation to be made from the same slide.

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Assessment of foetal maturity

by

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In many cases of pregnancy at risk, the foetus may have better chances in a nursery than in the uterus. The risk of death as a result of prematurity must obviously be avoided. The concept of maturity or « the state of being fully developed » ([1]), which had been instinctively associated with the size of the foetus, has now taken on a wider meaning.

It appears reasonable to admit that foetal maturity has been attained when the functional capacity of all the organs has reached the minimum level which allows the neonate to adapt to autonomous life.

The well-known factors which affect foetal growth are:

a) the maternal nutritional conditions;
b) placental sufficiency;
c) normal uterine blood flow;
d) the level of foetal insulin increase;

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