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The Stable Bubble Test

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SUMMARY

Idiopathic Respiratory Distress Syndrome (I.R.D.S.) occurs mainly in preterm babies (Halliday and McClure, 1976). The cause of the condition is a deficiency of surfactant in the fetal lung. (Avery and Mead, 1959). The condition, if untreated, is associated with high fetal mortality.

A simple test, The Bubble Stability Test, which can predict the possibility of I.R.D.S. occuring is now in use in the University Teaching Hospital, Lusaka. An analysis of the use of the Bubble Stability Test in fifty cases is presented.

In our series a positive Bubble Stability Test accurately predicted fetal lung maturity.

INTRODUCTION

Prematurity may be combated by increase in living standards and good antenatal care. Iatrogenic prematurity may be avoided if the obstetrician avoids delivery before thirty seven weeks. In some cases, delivery is necessary before full maturity is reached. By first testing for fetal lung maturity, the clinician may avoid delivering a baby whom he knows has insufficient surfactant at that time.

The surfactant present in amniotic fluid has been measured by estimation of the lecithin/sphingomyelin ratio (Whitfield et al, 1972) and measurement of the total lecithin concentration (Bhagwanai et al, 1972). Both tests are expensive and time consuming.

Clements and his colleagues (1972) described a simple, rapid test, the stable bubble test or 'shake' test. This test gives a semi-quantitative measurement of surfactant present in the liquor amnii, and reliably predicts the risk of I.R.D.S. occurring in the new born.

MATERIALS AND METHODS

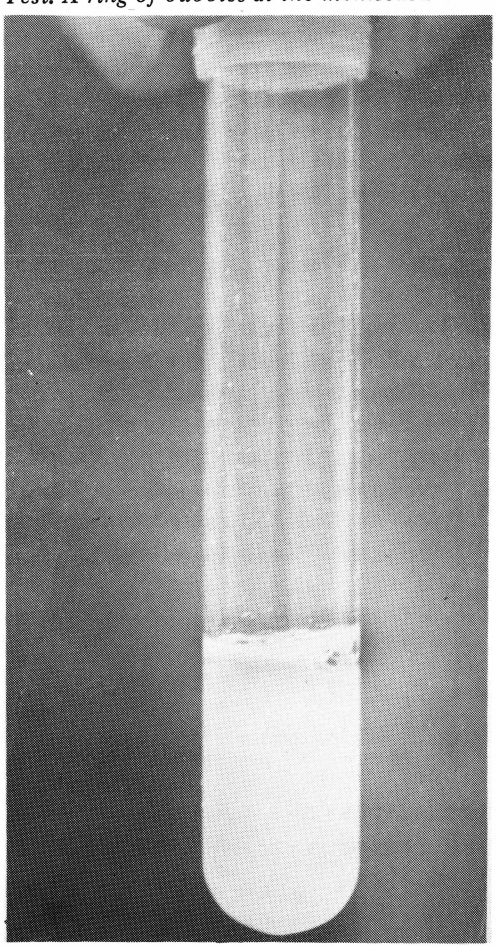
The Bubble Stability Test is a simple test and no sophisticated equipment is necessary for its performance. The method used follows Whitfield & Sproule (1974).

A sample of amniotic fluid is obtained by amniocentesis via suprapubic tap after bladder emptying and displacement of the fetal head, if necessary.

To 0.5ml of the amniotic fluid is added 0.5ml. of 0.9% saline. One ml. of 95% ethanol is then added to the test tube containing the amniotic fluid/saline mixture. A stopper is placed in the test tube which is then vigorously shaken for fifteen seconds, after which the tube is placed vertically in a rack. Following a lapse of fifteen minutes the presence or absence of bubbles at the meniscus is noted. A complete or incomplete ring of bubbles is taken as a positive test. Absence of bubbles denotes a negative test

FIGURE

The Photographic insert illustrates a positive Bubble Test. A ring of bubbles at the meniscusis noted



indicating that insufficient surfactant is present in the sample. The test may be repeated again in 48-72 hours if deemed necessary.

Precautions

Amniotic fluid contaminated by blood, soapy solutions, meconium or vaginal secretions should be discarded as these contaminants may give false positive results.

Saline from rubber stoppered infusion bottles often contains substances which form or interfere with formation of surface films. The 0.9% saline should be made up from reagent grade sodium chloride and distilled water, or if commercial isotonic saline is used, it should be supplied in screw top bottles.

The 95% ethanol is prepared from absolute ethanol by the addition of 10ml of distilled water to 190ml of absolute ethanol. The final concentration of ethanol in the solution is critical, and the stock bottle is kept tightly closed, except during pipetting, as this concentration of alcohol is hygroscopic.

The glass tubes, which measure 8–14mm x 100mm should only be cleaned with a dichromic acid solution and then rinsed six times. Dichromic acid is constituted by adding 10 grammes of Potassium Dichromate, 25mls. concentrated sulphuric acid and 75mls distilled water. All remnants of soap, serum, or biological fluids which might produce foam should be removed during cleaning.

Samples

Amniotic fluid samples were taken from fifty patients all of whom were delivered within 48–72 hours. The Bubbles Shake Test was performed and recorded. All infants tested were followed up, their weight and general maturity noted.

The babies were observed specifically for the onset of respiratory problems.

The duration of pregnancy in 32 of the patients was unknown. There were 5 twin pregnancies, 5 Elective Caesarean Sections and 5 cases of Hypertension. Three pregnancies were thought to have features of dysmaturity.

TABLE I

Pregnancy Complications	No.	IRDS
Twins	5	2
Hypertension	5	0
Suspected Dysmaturity	3	0
Unknown Dates	32	0
Bad Obstetric History	1	1

Results

The BST was negative in 4 cases. In two of these the infants weighed 1200 and 1000G respectively and both succumbed to IRDS and immaturity. The third baby weighing 2100 Grammes developed mild IRDS. The 4th baby who weighed 2400 grammes was unaffected. Of these four patients two were in labour and the other two went into labour within forty eight hours after testing.

The BST was positive in 6 cases in infants whose weight ranged from 1120 grammes to 2000g. Babies with a positive BST did not develop IRDS. Twenty three of the babies weighed less than 2,500 grammes at delivery. Eight weighed less than 2000 grammes. The six babies in this group with positive B.S.T.s. survived.

TABLE II

No. of Babies.	Weight (Grammes)	B.S.T.	IRDS
8	1000–2000	6 +ve	No
		2 -ve	Both (died)
15	2000–2500	13 +ve	No
		1 -ve	Mild
		1 -ve	Unaffected
32	2500–4200	All positive	No
TOTAL		4 -ve	3IRDS
55		51 +ve	No IRDS

From Table II it can be seen that eight of the babies weighed between 1000 and 2000 grammes. Two developed IRDS and died. Fifteen babies weighed between 2000 and 2500 grammes. One of these babies developed mild IRDS. Twenty-seven babies weighed over 2500 grammes and none developed IRDS.

Of the 50 patients tested only eighteen knew the date of their last menstrual period. This group delivered 5 premature, 3 dysmature and 10 mature infants.

Table 3 illustrates the weight, BST, and outcome of babies weighing less than 2000 grammes.

DISCUSSION

There are three successive phases of lung development (Charnock & Doe Roshuk 1973). During phase one, the bronchial tree is formed from the lung bud of the endodermal tube. The Respiratory bronchioles develop in the intermediate phase. Begin-

TABLE III

BABIES UNDER 2000 Grammes

(Grammes) Weight	B S T	I R D S	Survival
1000	- ve	Yes	No
1120	+ ve	No	Yes
1200	- ve	Yes	No
1600	+ ve	No	Yes
1700	+ ve	No	Yes
1720	+ ve	No	Yes
1740	+ ve	No	Yes
1860	+ ve	No	Yes

ning at twenty-four weeks and continuing into post natal life, the third phase is concerned with formation of the alveolar ducts and sacs, differentiation of the alveolar lining membrane into type 1 and 2 pneumocytes, and the production of surface active material by the type 2 cells.

The active components of this surface active material are phospholipids, maninly lecithin, (Gluck 1967) which increase with advancing gestation. Lecithin synthesis occurs by two separate pathways which differ in their developmental time table, molecular structure, and relative stability.

Pathway 1 is the formation of Dipalmitoyl (x Palmitic: B Palmitic lecithin). This is the active pathway from thirty four weeks. Pathway 2 is the formation of x palmitic-B myristic lecithin. This is the main pathway before the active terminal phase of pathway 1 occurs. Although only produced in very small amounts, pathway 2 enables some very premature babies to achieve and maintain adequate lung expansion. The early pathway may be inhibited by such factors as hypoxia, hypercapnia, acidosis, and hypothermia.

Because of fetal 'breathing' in utero, fluid drifts from the fetal lung to the amniotic fluid. Surfactant is carried in suspension in the fluid and if measured, should reflect its availability at the alveolar surfaces and thus, the potential stability of the alveolar structure. To avoid the relatively slow, costly and involved technique of phospholipid analysis, Clements et al, (1972), developed a cheap, quick bedside test, which is sufficiently sensitive and reliable for clinical use.

The rationale of the test is based on the ability of pulmonary surfactant to form highly stable surface films which can support the structure of a foam for relatively long periods. Other substances which can form stable foam in the amniotic fluid are excluded from the surface films by the non-foaming competitive surfactant, ethanol.

If amniotic fluid is mixed with ethanol, the system is poised so as to reveal the pulmonary surfactant present in the specimen when it is shaken with air to generate a foam.

In discussing the reliability of any measurement of surfactant in liquor amnii in the prediction of IRDS, allowance must be made for the occurrence of R.D.S., in the presence of satisfactory amounts of surfactant in maternal diabetes (Whitfield et al, 1973), and severe rhesus iso-immunisation (Whitfield et al, 1972). Delivery by caesarean section may increase the risk of I.R.D.S., and it is noted that I.R.D.S. is more common in boys. Polyhydramnios has been found to affect the reliability of predictions based on total lecithin concentration and the stable bubble test, but not those based on the Lecithin-Sphingomyelin ratio (Gerbie & Boehm 1973).

The low level of I.R.D.S. could mean that a good proportion of the low birthweight infants were dysmature. We suspect in retrospect that twelve of the infants below 2,500 grammes and six infants above 2,500 were dysmature.

The surfactant bubble or shake test, like the L/S ratio promises to be helpful in timing elective interruption of pregnancy, and in predicting the clinical course of the infant in premature labour.

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