MATERIAL AND METHODS

Population for study included infants delivered at Department of Obstetrics and Gynaecology, M.L.B. Medical College, Hospital, Jhansi. Newborns were divided into two groups (I and II) after taking careful antenatal history, examining mother for blood pressure, oedema, proteinuria, and conducting investigations to confirm diagnosis of toxemia of pregnancy.

Group I : It consisted of control cases delivered to healthy mothers.

Group II : It consisted of babies born to mothers fulfilling the criteria of the toxæmia of pregnancy.

In these mothers hypertension was pregnancy induced only. Placentae were examined for abnormality like infarction, haematoma, retroplacental clots and weighed after trimming the membranes and cutting the cord uniformly 10 cm away from placenta. If necessary for morphological examination confirmation, placenta were preserved in 10% buffered saline.

Haematological studies of cord blood of newborns were done in the Department of Pathology. Counting of cells was done with the help of Neubaur's chamber. Total Hb% with the help of colorimeter and foetal Hb% by alkaline denaturation method were done.

Weight of newborns and placenta was obtained from a weighing scale accurate to 50 gm. Crown Heel
length measurements in centimeters were done with the help of infantometer. Head circumference was measured in centimeter by measuring tape.

Thorough clinical examination was done in each case to exclude the possibility of congenital infections or anomalies.

Assessment of gestational age was done by using the physical and neurological characteristics laid down by Dubowitz et al (1970). The neurological characteristics were scored from 0-5, while 11 physical characteristics were scored from 0-4 in a pre-designed proforma and conversion of total score into gestational age was done by using the formula:

\[
\text{Period of gestation} = \text{Total score} \times 2642 + 24.595
\]

**COLLECTION OF BLOOD SAMPLES**

For the estimation of total Hb%, foetal Hb%, total platelet count, total leucocyte count, cord blood was collected in EDTA vials from the cut end of the umbilical cord from the maternal side.

**METHOD OF ESTIMATION OF TOTAL Hb%**

Range of Hb read 0-20gm/100 ml. Unit is based on the principle of converting Hb to cyenometh-haemoglobin and measuring its absorbance at 540 nm with respect to drabkin solution.
Dilution used is 1 : 25 (20 ul blood and 5 ml drabkin solution). Wait for 10 minutes. Now method of operation test sample is ready.

**Method of Operation**

1. Connect the unit to 230 V main socket and turn on allow 15 minutes warp up time.
2. Insert a test tube with drabkin and adjust set low for zero read out on the panel.
3. Press 'press to calibrate' and adjust set high for calibrated value specified. Unit is now ready for use.
4. Remove test tube with drabkins.

Insert samples and take reading one after other.

Ensure that the blood cover is closed while taking the sample.

**ESTIMATION OF FOETAL Hb%**

Hb-F and Hb Bart's are remarkable in that they are relatively much more resistant to denaturation by alkali than other types of haemoglobin. The rate of alkali denaturation has thus been employed widely as a test for the presence of Hb-F and as a means of estimating the amount of Hb-F in mixtures of haemoglobin, as for instance in cord blood.

Hb-F can be measured by the 1-min denaturation technique of Singer, Chefnoff and Singer, a simple
estimation of wide applicability but not capable of measuring accurately \( \text{Hb-F} \) when present in small amount i.e. \( \leq 4\%\).

**The Alkali Denaturation Technique**

Of Singer, Chefnoiff and Singer:

**Principle**

The haemoglobin solution under test is added to alkali and after exactly 1 minute the denaturation is arrested by the addition of 50% ammonium sulphate in acid solution. The amount of undenatured haemoglobin is measured photometrically or spectrophotometrically.

**Reagents:**

- Alkali - 0.083 N KOH or 0.083 N NaOH
- Precipitating solution - 50% saturated \((\text{NH}_4)_2 \text{SO}_4\) - 800 ml
- 10 N - HCl - 2 ml.

**Method**

32 ml of the alkali solution is placed in a test tube at room temperature or preferably in a water bath at 20\(^\circ\)C. 0.2 ml of the Haemoglobin solution - of concentration 10 g/100 ml is then pipetted into the alkali, mixed without delay and a stop watch is started. After exactly 1 minute 6.8 ml of the precipitating solution is added and after inverting the tube several times the mixture is filtered.

If the filtrate appears colourless there is little or no undenatured haemoglobin present. If not
colourless, the amount of pigment may be estimated by comparing it with a standard made by adding 0.1 ml of the original haemoglobin solution to 5 ml of 0.4 ml/l ammonia. This gives a standard equivalent to 100% Hb. If a photoelectric colorimeter and Ilford 625 filter are used for the measurements, it is wise to dilute this standard, e.g. 1 in 5 or 1 in 10, so as to match closely the colour of the test sample.

**LEUCOCYTE COUNT**

Make 1 in 20 dilution of blood by adding 0.02 ml of blood to 0.38 ml of diluting fluid in a 75 x 10 mm tube. Mix the suspension by rotating in a cell suspension. Mix it for at least 1 minute. Fill the Neubauer chamber by means of a pasteur pipette or stout glass capillary, as for red cell counts.

The red cells are lysed by the diluting fluid but leucocytes remain intact. View the preparation using a 4 m objective and x 6 ye piece count. The number of leucocytes in the four corner squares.

**Calculation**

\[
\frac{N}{0.4} \times 20 = N \times 50 \text{ WBC/mm}^3
\]

**PLATELET COUT**

The diluent consist of 1% aqueous ammonium oxalate in which the red cells are lysed. The method is recommended in preference to that using formal citrate
as diluent which leaves the red cells intact and is more likely to give incorrect results when the platelet count is low.

**Reagent:**

1% ammonium oxalate (1 gm in 100 ml of distilled water).

**Method**

Make 1:100 dilution of blood in the diluent by adding 0.02 ml of blood to 2 ml of diluent, mix it.

Fill Neubauer chamber with the suspension, using a stout glass capillary. Examine the preparation with 4 mm objective × 6 or × 10 eye piece. The platelet appear under ordinary illumination as small highly refractile particle.

The number of platelets in one or more areas of 1 mm² (or 0.1 mm³ volume) should be counted. The total number of platelet counted should always exceed 200.

**Calculation**

\[ N \times 10 \times 100 \text{ dilution} \]

\[ = N \times 1000/\text{mm}^3 \]