INTRODUCTION

The birth of a baby is a confluence of multiple events. This is a stage of horizon when a foetus is ready to take over the responsibilities of an independent existence from its parasitic environment. During this period, profound changes take place in the internal biological processes so as to cope up with the demands of a separate existence. Within few minutes it has to open up a new thoracic circulation and also has to initiate its unskilled respiratory apparatus for supply of oxygen. Formation of some of the new enzymes and regulatory mechanisms which were not developed during the parasitic existence, now has to be initiated during this process of adjustments and adaptation. 'This is a time during which life is more dynamic than static, for in no other equally brief span of existence do such profound alterations and adjustments occur as in the weeks, or even the days or hours following birth' (Smith, 1959).

For all vital processes of anabolic and catabolic in nature, energy is required which is ultimately derived from glucose and its intermediate metabolic products. The main stores of energy are glycogen (Shelley, 1964) and fat (Widdowson and Spray, 1951) and a full-term normal infant is well endowed with both. Not all organs of the body can store energy and so the infant will have to keep the levels of

energy producing substances circulating at a level high enough for its needs.

The overall low levels of fasting blood sugar in the newborn infants as compared to that in the adults have been extensively documented. Moreover, still lower blood sugar levels have frequently been reported in the infants born with special problems such as low birth weight, maternal diabetes, maternal toxaemia, smaller of the twins, primary cerebral damage, polycythemia etc. A great deal of speculation has been entangled around the homeostatic mechanisms responsible for the low blood sugar levels in the neonatal infants. This includes increased insulin sensitivity (Hartman and Jaudon, 1937; Hartman, 1955), increased insulin secretion (Kohler, 1932; Ketteringham and Austin, 1939; Watcher, 1949; Desmond, 1953), hepatic immaturity (Van Crevald, 1929; Pedersen, 1952), adrenal insufficiency (Farquhar, 1954), capacities of the organs to utilize glucose independent of insulin (Cornblath, Wybregt and Baens, 1963), inadequate glycogen stores of the liver (Shelley and Neligan, 1966) and the interplay between insulin and its antagonists. However, the problem regarding the observations of low blood sugar in neonatal infants is not satisfactorily solved and the available data do not permit a convincing conclusion.

The concentration of blood glucose in the fasting state in a normal individual depends on the balance between two

general processes viz. (i) the rate of glucose entrance into and (ii) the rate of its removal from the blood stream. The former process includes glycogenolysis and gluconeogenesis, while the later includes glucose utilization and its storage in various body organs (glucose disappearance). In an adult individual glucose, free fatty acids (FFA), insulin, glucocorticoids, glucagon, growth hormone, adrenalin and thyroxin interact and play important roles in the regulation of energy metabolism.

Due emphasis has been given to the prerequisite factors affecting the blood glucose levels during the early neonatal period. This includes the duration of the gestational period (prematurity), duration of labour, drugs administered during labour period, mode of delivery, age of the infant and ante-natal conditions of the mother, like toxaemia of pregnancy and diabetes etc. Moreover, Cornblath et al. (1963) advocated the influence of pH, ketosis, body temperature, degree of oxygenation and concentration of fatty acids on the metabolism of glucose. However, these factors have not been fully explored except in some of the previous reports.

Considering the above factors, the present study has been designed to get an overall picture of the mechanisms by which the blood glucose level is regulated - the process of 'entry' and rate of glucose 'disappearance'. These have been carried out by administration of glucagon and glucose on two different occasions.

This study can be resolved into the following stages:

(i) To assess the probable period of stabilization for the blood glucose levels in the normal full-term infants.

(ii) To report the behaviour of glucose after glucagon administration on the first and eighth days of life in the normal full-term infants. This would give an idea about the availability of the hepatic glycogen stores and glucose output, integrity of the glycogenolytic enzymes and its pathways and the rate of disappearance of glucose from the circulating blood (Peripheral utilization).

The behaviour of plasma glucose after oral glucose administration in the full-term normal infants on the second and the eighth days would give an idea of absorption, glycogenesis and peripheral utilization of glucose.

(iii) To report the behaviour of plasma inorganic phosphorus, potassium, urea and amino acid nitrogen after glucagon administration on the first and eighth days and that of phosphorus and potassium after oral glucose load on the second and on the eighth days of life.

The changes in plasma inorganic phosphorus and potassium after glucagon as well as glucose administration would indicate the extent of

glycogenolysis and glycolysis, while the changes in the concentrations of urea and amino acid nitrogen after glucagon administration would give an idea about the rate of gluconeogenesis.

(iv) To report the behaviour of the above parameters in various groups of infants viz. healthy premature infants, healthy low birth weight infants, infants delivered by forceps application, infants delivered by Caesarean section and infants with maternal toxaemia.

(v) To study and compare the results obtained in the full-term normal infants with those of the other groups.

A complete statistical evaluation is an unattainable perfection during this age period when the changes in the individual infants show considerably wide variations. However, the trends seen in the mean values of each group of infants appear to be meaningful and are utilized to illustrate the changes.

CLINICAL MATERIAL

The clinical material in the present study consists of infants delivered at Shree Sayaji General Hospital, Baroda.

Twenty three full-term normal infants (15 males and eight females) have been studied to assess the probable period of stabilization of the plasma glucose levels. The record of their sex, weight, maturity score, condition at birth, progress, length of labour etc. were noted. Infants who had their birth weight over 2500 G and their gestational age more than 36 weeks were included in this group. These infants were delivered by spontaneous vaginal delivery and showed normal breathing, colour and satisfactory general behaviour. All had Apgar score more than seven at five minutes. Clinical details of their mothers regarding the type of delivery and maternal obstetric history were recorded.

No antenatal as well as postnatal abnormalities were noted and their deliveries were uneventful. Clinical data of these infants as well as their mothers are summarised in Appendix I.

One hundred and thirty nine glucagon tolerance tests and 135 glucose tolerance tests were carried out on 79 newborn infants (41 males, 38 females), for the study of carbohydrate metabolism in the neonatal infants. This included 17 full-term normal infants, 15 healthy premature infants, 15 healthy low birth weight infants, eight infants delivered by

forceps application, ten infants delivered by Caesarean section, ten infants with maternal toxaemia, three infants with maternal diabetes and one premature infant who showed the signs of sclerema on the fourth day of life.

The criteria of all the 17 full-term normal infants included in the present study were the same as those mentioned above.

Controversy still persists amongst the different authors within the developing countries regarding the differentiation of low birth weight infants and premature infants. Various authors have suggested different weight criteria for low birth weight infants. Criteria of maturity also differ from author to author, which are either based on various physical and neurological responses or on gestational age. The gestational age is likely to be incorrect for the obvious reasons. Considering the difficulties in obtaining the correct informations regarding the gestational age, the below mentioned criteria were followed. (i) Birth weight: 'The expert committee on Maternity and Child Health of the World Health Organisation in 1961, classified all infants with birth weight less than 2500 G as low birth weight infants and infants born at any time before 37 weeks as premature or mature. (ii) Maturity score: It has been a practice to record maturity score of almost all infants delivered in this institution by a scoring system suggested by Chikermane, Majmudar and Shah (1969), which is based on various physical

and neurological criteria. The maximum score is 20. Infants who have their maturity score below 14 are termed as premature infants and those who have 14 or more are termed as low birth weight infants (in the infants whose birth weights are less than 2500 G).

Fifteen healthy premature infants, who could satisfy the above mentioned criteria and who were delivered by spontaneous vaginal route were included in the premature group of infants.

Infants included in the low birth weight group satisfied the above mentioned criteria i.e. they all had their birth weight less than 2500 G, gestational ages between 37 weeks or more and maturity scores were more than 13. All these infants were delivered spontaneously by vaginal route. Their mothers had no other abnormalities and the deliveries were uneventful.

This study includes eight infants delivered by forceps application. All the infants in this group had their birth weight more than 2500 G and their gestational age were 37 weeks or more. Only local anaesthesia was used. One mother received 5% glucose infusion during her labour period (F 3). The application of the forceps were advised either for foetal distress or for a prolonged duration of second stage of labour.

The group of the infants delivered by Caesarean section includes ten cases. Eight infants in this group were

full-term infants. Biochemical data of the remaining two premature infants (C 9 and C 10) are not included for the statistical evaluation. All the mothers undergoing Caesarean section received 5% glucose (or glucose saline) infusions either during their labour period and/or during surgery. Caesarean sections were carried out under general anaesthesia. Out of the 10 cases, only one is an elective Caesarean.

This study includes ten infants with maternal toxaemia. Seven of these infants had their birth weight 2500 G and more and their gestational age were 37 weeks or more. Out of these seven infants, two were delivered by forceps application. The remaining three infants satisfied the criteria of prematurity (premature infants with maternal toxaemia). Statistical data of only two sub-groups viz. full-term infants with maternal toxaemia (T 1 to T 5) and premature infants with maternal toxaemia (T 8 to T 10) have been presented.

Only three infants were followed in the group of infants with maternal diabetes.

The clinical data of all the infants and their mothers are summarised in Appendix I.

COLLECTION OF MATERIAL

Twenty three full-term normal infants, who were studied for the assessment of the period of stabilization of plasma glucose levels, were isolated from their mothers for first 24 hours after birth and were kept under the supervision of the nursing staff. All the feeds except plain sterile water were withheld for the first 24 hours of life and then for at least three and a half hours on the subsequent days before collection of the blood specimen. Blood samples were collected during the first hour, at three, six, 12 and 24 hours and also on the third, fourth, fifth, sixth and seventh day of life.

A thin film of sterilised paraffin wax was applied on the heel side (Pildes, Hart, Warrner and Cornblath, 1969 a) before pricking, to facilitate the formation of an adequate size of the blood drop, which was then directly received into a dry heparinised Widal tube. The tube was shaken intermitently during the process of blood collection to give an anticoagulant effect. The tube containing blood sample was centrifugalised at 3000 r.p.m. for 5 minutes and a measured quantity (0.1 ml.) of the separated plasma was transferred to the protein precipitant (0.34 M perchloric acid). The whole procedure was completed within ten minutes. Protein-free clear supernatants were preserved in the refrigerator between 2° to 6° C till their analysis for

glucose on the following day.

For the glucagon responses on the first day, the basal sample was collected within two to three hours of life before any feeding was started, while on the eighth day, it was collected after three and a half hours fast. A dose of 30 µg. glucagon per kilogram (kg.) body weight (300 µg. glucagon per kg. in the infants delivered by Caesarean section on the first day) was administered intramuscularly (i.m.) on both first and eighth days. The blood samples were collected at 20, 40, 60, 120 and 150 minutes after glucagon administration. Clear protein-free supernatants and the remaining plasma samples were preserved in glass-stoppered tubes between 2° to 6° C in the refrigerator till they were analysed for glucose, inorganic phosphorus, potassium, urea and total amino acid nitrogen on the following day.

The same infants were followed for oral glucose tolerance tests on the second and eighth days of life. On the second day, all the feeds were withheld as mentioned above before the collection of the basal sample. On the eighth day, the sample collected at 150 minutes after glucagon administration represented the basal sample. Glucose 2.5 G/kg. body weight was administered in the form of 25% glucose solution (sterile) by gavage tube. Blood specimen were collected at the interval of 30 minutes for

150 minutes. Protein-free supernatant and the remaining plasma samples were preserved in the refrigerator as shown above. The samples were analysed for plasma glucose, inorganic phosphorus and potassium concentrations.

ANALYTICAL METHODS

The recent development of ultra-micromethods in the study of neonatal biochemistry has helped to perform blood chemistry studies on small samples of capillary blood obtained from a heel prick. The laboratory methods used for this study are standard methods modified to the requirements of the available samples. The total volumes were kept throughout as small as possible avoiding work with highly diluted solutions. Beckman spinco polythelene (with water repellent surface) pipettes were used in this work. At frequent intervals, the pipettes were filled with a fresh solution of 1% pepsin in 0.1 N hydrochloric acid and were left overnight to remove any thin invisible coating of protein.

For colorimetry, a spectrophotometer Unichem S.P.600 and Evan Electroseleium Limited (E.E.L.) titrator unit with 10 mm. light path were used. The minimum quantity of the fluid required for Unichem S.P.600 was 0.5 ml. (micro attachment) and for E.E.L. titrator unit was 2.0 ml. The methods used in this study are suitable for very small amounts of samples and the biochemical constituents were analysed using 20 to 50 µl. of the specimen.

Standards of low and high concentrations were included in all the batches of the samples. At times, the quality control sera were also included in some of the batches, the

results of which were found satisfactory.

American Diabetes Association in 1968, recommended the use of plasma or serum rather than whole blood specimen, for glucose determinations. This becomes necessary in a study like the present series, where the comparisons of the glucose and other parameters at two different occasions (at an interval of one week) comprised the fundamental basis for evaluation of the results. This would exclude the possible variations due to the fluctuations in the haematocrit levels during the early neonatal period (Acharya, 1962; Haworth, Dilling, and Younoszai, 1967).

Plasma glucose was determined by Glucose oxidase method (Bergmayer, 1968). Perchloric acid used in this method acts both as a protein precipitant and as a glucose preservative. Glucose is oxidised to gluconic acid with the gluconolactone as an intermediate. The hydrogen peroxide formed during this reaction is broken down to water and oxygen by peroxidase in presence of an oxygen acceptor (o-dianisidine) which is converted to a coloured compound. The colour is fixed by the addition of sulphuric acid (50% w/w). The final pink colour is read colorimetrically.

Plasma inorganic phosphorus was determined by the method of Natelson (1961) using 1,2 aminonapthol 4-sulphonic acid as a reducing agent.

Plasma potassium was determined by E.E.L. flame photometer using external standards. 20 µl. plasma was

diluted to 2.0 ml. with double distilled water.

Plasma urea was estimated by Natelson's method using tungstic acid supernatant. Diacetyl monoxime was used to react with urea in strong acid solution and the yellow colour developed is read colorimetrically (Natelson, 1961).

Plasma total amino acid nitrogen (TAN) was determined by Natelson's technique (Natelson, 1961). Buffered ninhydrine reagent is allowed to react on the protein-free supernatant to produce reddish-violet colour which is then estimated colorimetrically.