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Effects of Aspartic Acid, Orotic Acid, and Glucose on Serum Bilirubin Concentrations in Infants Born before Term

The transient unconjugated hyperbilirubinaemia of the newborn infant, most marked in those born before term, is associated with a limited ability of the liver to form bilirubin glucuronide (Vest, 1958). Bilirubin glucuronide is formed by the transfer of glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) in the presence of the microsomal enzyme UDP-glucuronyltransferase (uridine diphosphate glucuronate glucuronyltransferase, E.C.2.1.17). On the basis of studies in experimental animals (Dutton, 1959), it is usually considered that deficiency of this enzyme is the main aetiological factor in causing hyperbilirubinaemia, but it has not been possible to confirm this hypothesis since a stable purified preparation of this enzyme has not been studied (Mowat and Arias, 1970).

An alternative hypothesis, that the hyperbilirubinaemia is due to a relative deficiency of UDPGA, correctable by ingesting uridine precursors such as aspartic acid and orotic acid, prompted the study of Matsuda and Shirahata (1966) who showed that in healthy term infants both aspartic acid and orotic acid lower serum bilirubin concentrations. The present observations were made on pre-term infants, in whom 'physiological' jaundice may be sufficiently severe to cause kernicterus, in order to determine whether the action of these agents

might be of value in preventing serious hyperbilirubinaemia.

Methods

Observations were made on 34 healthy pre-term infants of known gestational age. All were without haemolytic disease, infection, respiratory distress, or congenital abnormality, and none was receiving drugs.

The infants, matched for sex, were allocated to three main groups of equal size who received orotic acid, glucose, or aspartic acid in equimolecular amounts in a double-blind fashion. Each group was subdivided by gestational age into three equal groups of 31 to 33 weeks; 34 to 36 weeks; and 37 to 39 weeks' gestation. In the most immature group of infants receiving orotic acid and glucose, observations on one female infant were incomplete and therefore not considered. Since gestational age was calculated from the date of onset of the mother's last menstrual period, only infants born to mothers who were certain of this date and whose menstrual cycles were regular were included in the study.

Infants in the study were fed according to a standardized feeding regimen involving 10% glucose feeds in the first 24 hours of life and the introduction of half-strength half-cream dried milk (Cow and Gate) within 36 to 48 hours followed by full-strength half-cream milk 48 hours later. Solutions of orotic acid, glucose, and aspartic acid (as magnesium aspartate) were made up to contain 130 mg, 125 mg, and 100 mg/per 12.5 ml, respectively. These were given twice daily with feeds, starting within 24 hours of birth, according to the following weight-based dosage schedule: 1.0–1.5 kg, 7.5 ml; 1.5–2.0 kg, 10.0 ml; 2.0–2.5 kg, 12.5 ml for a total of 12 doses.

Serum bilirubin levels were determined on the 2nd, 4th, 6th, and 8th day by the method of Lathe and Ruthven (1958). All values reported are of indirect-reacting bilirubin.

Results

The mean serum bilirubin values determined 2, 4, 6, and 8 days after birth follow a similar pattern in all three treatment groups, with a wide overlap in values between the groups. In female infants values were lower than in males, but this difference did not reach statistical significance (Table). Maximum serum bilirubin levels were found in the glucose-treated group on day 2 in females and on day 4 in males; on day 6 in the group receiving orotic acid; and on day 2 in those receiving aspartic acid. In contrast to the findings in other groups, mean serum bilirubin levels in male infants treated with aspartic acid fell from the highest value on the 2nd day. In this group of infants maximum serum bilirubin levels on days 4, 6, and 8 compared with the level on day 2, were significantly lower ($p < 0.01$) than those in other groups. There were no other significant differences in serum bilirubin levels.

TABLE
Serum Bilirubin Concentrations (mean $\pm 1SD$, mg/100 ml) in Each Treatment Group

Treatment	No. of Infants	Day 2	Day 4	Day 6	Day 8
Orotic acid	6 M	6.9 $\pm$ 3.2	11.8 $\pm$ 5.9	11.0 $\pm$ 6.5	4.3 $\pm$ 2.5
	5 F	4.9 $\pm$ 2.5	7.8 $\pm$ 4.9	7.3 $\pm$ 5.5	3.7 $\pm$ 4.2
Glucose	6 M	6.8 $\pm$ 3.3	10.1 $\pm$ 4.1	9.2 $\pm$ 3.5	7.5 $\pm$ 4.2
	5 F	7.2 $\pm$ 2.2	7.3 $\pm$ 2.0	5.7 $\pm$ 2.2	5.2 $\pm$ 3.1
Aspartate	6 M	8.7 $\pm$ 3.8	8.7 $\pm$ 3.3	8.6 $\pm$ 3.5	6.2 $\pm$ 3.5
	6 F	7.4 $\pm$ 3.8	8.7 $\pm$ 3.0	8.8 $\pm$ 4.1	6.8 $\pm$ 3.3

Weight loss at 1 week of age, expressed as a percentage of birthweight was less in the orotic acid treated group (mean 5.3%, range 0–11%) than in those receiving aspartic acid (mean 7.2%, range +1.6–15%) or glucose (mean 7.9%, range 1–16%). Maximum serum bilirubin levels were not related to weight loss at 1 week whether expressed in this fashion or in absolute amounts, nor were they closely related to the initial weight of the infant.

The infants were closely observed in an intensive care unit throughout the study but showed no adverse effects from these products. Of the 34 infants, 27 (80%) have now been examined at the age of 9 months. All are developing normally.

Discussion

These observations do not support the findings of Matsuda and Shirahata (1966) who in 60 term infants in Japan recorded that both aspartic acid and orotic acid significantly lowered serum bilirubin levels. In their report these authors do not indicate whether the infants studied were male or female, though, as found in this study, it has been reported that in males serum bilirubin levels are higher than in females (Trolle, 1965). A dosage schedule similar to ours was used in their study but they make no comment on dietary intake. The dose of aspartic acid used in this trial is equal to the anticipated daily intake of an infant fed on cow's milk, but the dose of orotic acid is 10 times that derived daily from milk. In spite of this no adverse effects were noted though this agent may cause adverse effects in rats but not necessarily in other species (Valli, Sarma, and Sarma, 1968).

This study does not refute the possibility that the administration of uridine precursors may enhance uridine formation in man, as they have been shown to do in experimental animals (Bresnick, Mayfield, and Mossé, 1968). Certainly they had no effect on serum bilirubin concentrations, but these are governed by many other factors. On the basis of evidence presented, neither aspartic acid nor orotic

acid is a useful therapeutic agent in the management of neonatal hyperbilirubinaemia.

Summary

In a double-blind trial, administration of uridine precursors (aspartic acid, orotic acid) did not affect the serum bilirubin levels in healthy preterm newborn infants.

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False Negative Screening Tests in Phenylketonuria

The routine screening of babies for phenylketonuria is now widely practised, using either a blood sample (Guthrie test) or a urine sample (paper chromatography or ferric chloride methods). The validity of the test depends on an adequate dietary protein intake, and a false negative may result if the