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Changes in the Auditory Brainstem Response Associated with Intravenous Infusion of Unconjugated Bilirubin into Infant Rhesus Monkeys

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ABSTRACT. The auditory brainstem response (ABR) was monitored in nine infant rhesus monkeys during the intravenous infusion of 50–168 mg/kg of unconjugated bilirubin. Sulfisoxazole (200 mg/kg) was sometimes given near the end of or just before the bilirubin infusion if no obvious ABR change had yet occurred. Five of the animals were term gestation, four were preterm, and they ranged from 1 to 40 days of age at the time of study. The three oldest term animals, studied at 20, 35 and 40 days of age, respectively, showed variable changes in the ABR waves during bilirubin infusion and these changes were not altered further by sulfisoxazole administration. The other two term infants, studied at 1 and 6 days of age, respectively, showed sulfisoxazole enhanced ABR wave latency increase and amplitude reduction followed by loss of the ABR. Both of these animals became apneic following ABR loss and eventually died. The ABR reappeared in one animal prior to death. Minimal gross and microscopic changes were present in the brain of the 6-day-old animal at autopsy. The four preterm animals all had a progressive wave amplitude decrease followed by loss of the ABR with bilirubin alone. These preterm animals were sacrificed shortly after the ABR loss with only one showing yellow staining of the basal ganglia at autopsy. The infant rhesus monkey may be a useful paradigm for bilirubin-induced ototoxicity as manifested by potentially reversible ABR changes. The changes are dependent on gestational and chronological age of the animal and appear to occur in the peripheral eighth nerve or cochlea as well as in brainstem pathways. (*Pediatr Res* 20: 511–515, 1986)

Abbreviation

ABR, auditory brainstem response

Hearing loss occurs in about half of the infants surviving bilirubin encephalopathy (1), and the ABR measured months or years following the bilirubin injury is often absent or present only at increased stimulus intensity suggesting auditory nerve injury (2, 3).

Recent reports have documented a variety of acute, usually reversible ABR wave latency changes, amplitude changes, and loss during neonatal hyperbilirubinemia. It is speculated that at least some of these changes represent reversible bilirubin toxicity, although the data are conflicting as to whether the toxicity is occurring primarily in brainstem pathways (4–7) or auditory nerve (8).

Since ABR changes following bilirubin toxicity cannot be demonstrated in the adult Gunn rat, the classical animal model for kernicterus (9, 10), we undertook this study using infant rhesus monkeys (11) to determine whether this animal might be suitable as a model for more detailed investigation of the ABR as a tool for evaluating bilirubin toxicity to the auditory pathway.

METHODS

Nine infant rhesus monkeys (*Macaca mulatta*) aged 1 to 40 days were studied at the California Primate Research Center. Four of the infants were delivered preterm at 0.8 gestation by cesarean section and tracheally intubated prior to clamping the umbilical cord. They were mechanically ventilated and cared for under an open warmer to prevent acidosis, asphyxia, and hypothermia. An umbilical artery catheter was inserted in these infants for blood gas and blood pressure monitoring as well as glucose and fluid administration. The initial arterial pH in all infants was greater than 7.25. The other five infants were delivered at term, one by cesarean section. The term animals were 1, 6, 20, 35, and 40 days of age, respectively, when studied, and received 0.1 mg/kg of oxymorphone intravenously as needed for sedation (usually every 2–3 h). The drug had no apparent effect on the ABR.

The ABRs were obtained using a Nicolet CA 1000, Nicolet Compact 4, or a Tracor-Northern signal averager. Needle electrodes were placed at the vertex and either over the mastoid behind the left ear or linked electrodes were placed in the posterior wall of the external auditory canals bilaterally. A ground was placed either on the left arm or in the mid-frontal region. Two thousand rarefaction click stimuli of 0.1 ms duration and frequency 11 or 33/s, were averaged. The latencies and amplitudes from baseline of waves I, II, and IV were measured (12, 13) as these waves were consistently present in all animals (Fig. 1). The IV–II peak to peak interval was used as a measure of brainstem conduction as wave II in the monkey is felt to arise from the extradural portion of the eight nerve (14). A change in latency or amplitude was considered significant if the linear regression plot of the variable against time had a slope which was significantly different from zero or if during the study the variable had changed on three consecutive ABR tracings by at least 2 SDs from the average of three to six baseline values. An average of 27 ABRs were obtained during the bilirubin infusion (range 8–60).

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Once ABRs between 35 and 95 dB were obtained to assure normal hearing and identify waves, the infant was monitored at 80 or 85 dB prior to (baseline values) and during bilirubin infusion. Bilirubin solutions of about 10 mg/ml were prepared by dissolving the appropriate amount of bilirubin (Sigma Chemical Co., St. Louis, MO, product B-4126) in 1.0 M NaOH, adjusting the pH to 8.3 to 8.5 with 1.0 M HCl, and diluting to the appropriate final volume with 10–20 ml of 0.75 mM NaCl containing 0.5 U/ml of sodium heparin. The bilirubin solution was protected from light, replaced with a fresh solution every 2 h, and was infused into a peripheral vein. A 10 to 20 mg/kg loading dose of bilirubin was given followed by a continuous or intermittent (every 15–30 min) infusion of the bilirubin solution until either a total dose of about 150 mg/kg was given (usually 3–4 h) or obvious ABR abnormalities developed. If no ABR changes were apparent, the infant was given 200 mg/kg of sulfisoxazole intravenously (15). Periodic blood samples were obtained from a site remote from the bilirubin administration site, for measurement of the total and direct serum bilirubin concentration and, in some animals, the serum albumin and unbound bilirubin concentrations (16, 17).

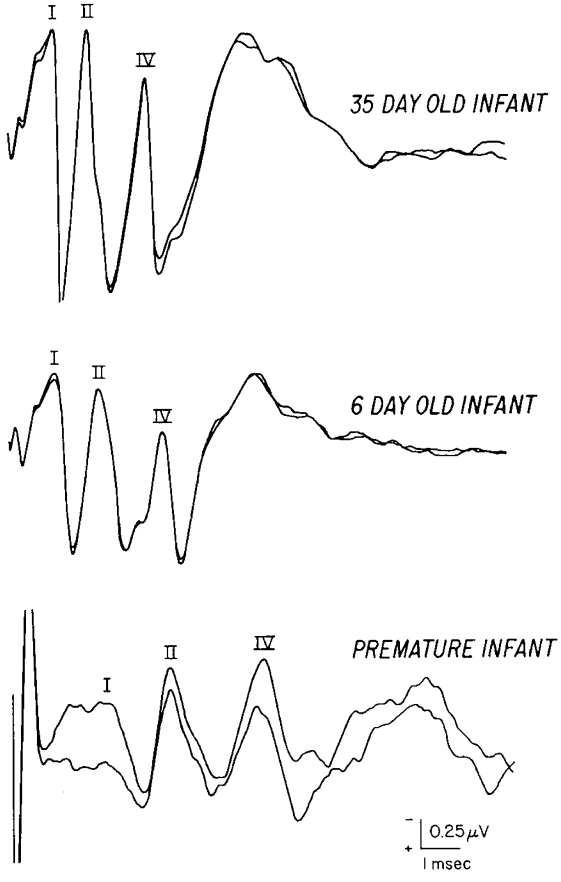

Fig. 1. Typical auditory brainstem response in term animals of different ages (35- and 6-day-old infants) and a premature infant at 1 day of age. Duplicate tracings are superimposed to verify the reproducibility of the tracing where possible.

Autopsies were performed on the preterm animals and on the term animal studied at 6 days of age. Brains were removed, fixed in formalin, stored in the dark, and grossly examined after 4–5 days. Since oxidation of bilirubin in the brain to colorless compounds may occur, selected tissue blocks were embedded in paraffin and sections stained with hematoxylin and eosin and luxol fast blue-cresyl violet to look for microscopic changes compatible with bilirubin toxicity (11).

RESULTS

The baseline ABRs showed wave I, II, and IV latencies and amplitudes which were dependent on both the gestational age and chronological age of the animals as has been described previously (13). The latencies were longer with smaller amplitudes in the younger animals (Fig. 1).

The ABR response to the infusion of bilirubin was also dependent on gestational age and age of the animal at the time of study. Three types of ABR responses to bilirubin were seen which allowed the animals to be divided into three groups. Group I (three oldest term animals) showed no ABR change or variable wave latency or wave amplitude changes following bilirubin and sulfisoxazole. Group 2 (two youngest term animals) showed increase in wave latency and reduction of wave amplitude followed by loss of ABR after bilirubin and sulfisoxazole. Group 3 (four preterm animals) showed primarily reduction in wave amplitude followed by loss of ABR during bilirubin administration. The ABR responses of the animals are summarized in Table 1.

In the three oldest animals (group 1) inconsistent latency and amplitude changes were found (Table 1). Mean serum bilirubin concentrations reached 79.8 mg/dl (SD ± 19.8 mg/dl). Direct bilirubin did not exceed 3 mg/dl. Hemolysis occured in one animal and they all became lethargic. All had recovered fully 48 h following the bilirubin infusion. Since no change in the IV–II ABR wave interval occurred in any of the animals, ABR changes

Table 1. *Summary of animals' ABR responses**

Group and gestation	Age of animal (days)	Total bilirubin (dosage (mg/kg)	Highest indirect serum bilirubin (mg/dl)	Serum albumin (g/dl)	Sulfisoxazole given	Wave Changes During Bilirubin Infusion				
						I Lat Amp	II Lat Amp	IV Lat Amp	IV-II Lat	Eventual ABR loss
1	20	158	>100	3.9	–	––	––	––	–	–
Term	35	106	79.2	ND	+	↑↓	↑↓	––	–	–
	40	166	60.3	ND	+	↑–	↑–	↑–	–	–
2	1	133	36.0	2.8	+	––	––	––	↑/S†	+/S
Term	6	168	76.2	3.8	+	–↓	–↓	–↓	↑/S†	+/S
3	1	90	33.0	2.7	–	–↓	–↓	–↓	–	+
Preterm	2	105	54.0	2.7	–	↑↓	↑↓	↑↓	–	+
	1	50	28.8	2.3	–	–↓	–↓	↑↓	–	+
	1	133	20.1	ND	–	–↓	–↓	–↓	–	+

* Lat, latency (ms); Amp, amplitude (μV); +, yes; –, no or no significant change; ND, not determined; Arrows, significant increase (↑) or decrease (↓).

† ABR change followed sulfisoxazole.

when they did occur were probably external to the brainstem in the cochlea or auditory nerve.

The animals in group 2 (term infants, studied at 1 and 6 days age) showed minimal ABR change during the bilirubin administration. Sulfisoxazole administration following the bilirubin in one animal and just before cessation of the bilirubin infusion in the other produced an initial latency increase in waves II and IV with decrease in amplitude of I, II, and IV and eventual loss of ABR waves (Fig. 2 and 3). The venous pH in both animals at this time was greater than 7.3. The IV–II interval increased as well suggesting intra- and extra-brainstem toxicity. Both animals lost the righting reflex and became apneic shortly after ABR loss and required resuscitation. Both eventually died. The 6 day old showed a 10-fold rise in unbound bilirubin and dramatic decrease in total serum bilirubin with sulfisoxazole (Fig. 2). He had a transient recovery of the ABR before dying from an aspirated feeding 20 h later (Fig. 2). Albumin administered to the infant did not acutely restore the ABR. The progressive latency and amplitude changes following sulfisoxazole are shown for the 1-day-old animal in Figure 3. The serum bilirubin in this animal prior to administration of sulfisoxazole was 36.0 mg/dl and the unbound bilirubin doubled with administration of the drug. We also monitored the infant using NMR (Oxford TMR 3500). A transient lowering of the ATP phosphate peak with increase in the inorganic phosphate peak was observed (Fig. 4).

The animals in group 3 (preterm, ventilation supported) began to show decreasing amplitude of all waves after a mean bilirubin dose of 84 mg/kg with subsequent ABR loss. It was not possible to determine whether the hair cell, auditory nerve, or both were the principal site of toxicity. The maximum serum bilirubin concentration ranged from 30 to 54 mg/dl. A typical response is shown in Figure 5. One of these animals showed a transient loss of ABR early in the bilirubin infusion associated with a plugged endotracheal tube. This resolved within a few minutes when ventilation was restored. All animals were sacrificed 2 h after the ABR loss. The brain of one of the preterm animals showed symmetrical yellow staining of the basal ganglia (globus pallidus and putamen). Minimal microscopic changes found in several instances included Alzheimer type II astrocytes in the cortex and basal ganglia. No significant brainstem changes were noted microscopically. Neuronal morphology was normal.

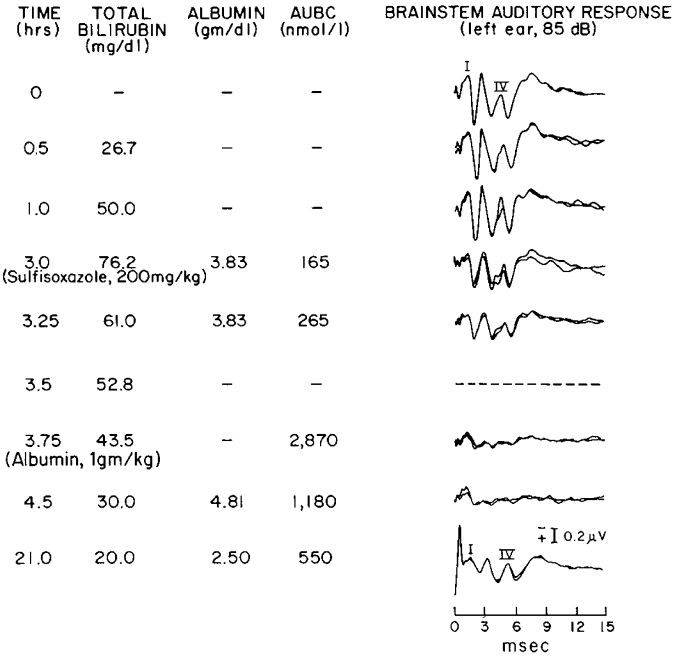

Fig. 2. ABR response, serum bilirubin, albumin, and unbound bilirubin (AUBC) in a 6-day-old term infant following administration of bilirubin and sulfisoxazole. Albumin was administered with no immediate effect after ABR wave loss. A vestigial wave I may be present in the penultimate tracing. 10 nmol/liter of unbound bilirubin is about 0.6 μg/dl.

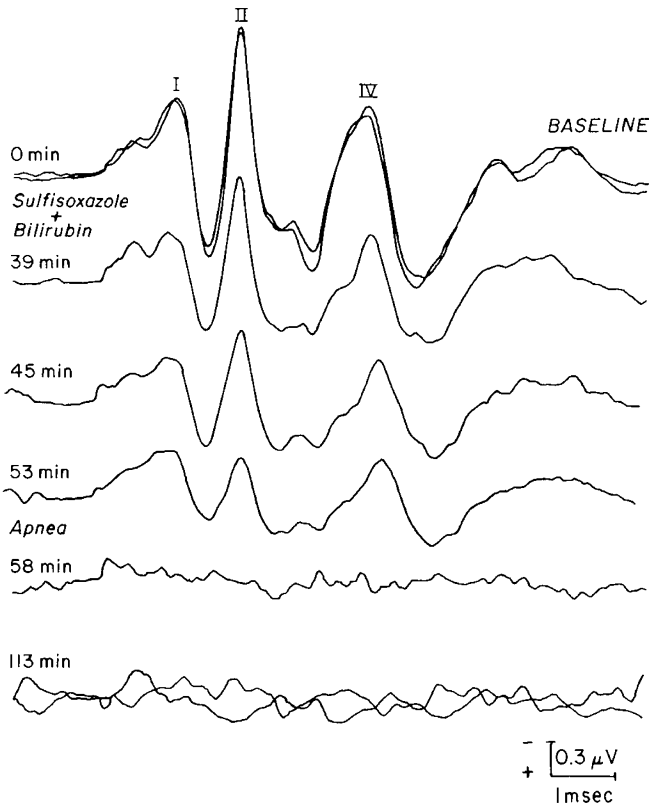

Fig. 3. ABR changes following sulfisoxazole in a 1-day-old term infant. The infant received about 10 mg/kg of bilirubin immediately after the sulfisoxazole. Increased latencies and reduced amplitudes of waves II and IV and increases in IV–II interval occurred before loss of ABR waves.

DISCUSSION

Clinical evaluation of the jaundiced newborn at risk for bilirubin encephalopathy continues to be a major problem in pediatrics (18). Since reversible ABR wave changes have been associated with hyperbilirubinemia in the human newborn (4, 7, 8), it is possible that ABR monitoring of the jaundiced infant may prove to be an important clinical tool for detecting early, reversible bilirubin injury. Although it is not clearly established that these bilirubin-associated ABR changes indicate a need for more aggressive treatment of the jaundice, the continued association of sensorineural hearing loss with hyperbilirubinemia, particularly in the premature infant, indicates a need for further investigation in this area (19, 20). The paradigm in this study appears useful in this regard.

While the susceptibility of the animals to bilirubin ototoxicity as manifested by ABR changes was variable and clearly related to maturational factors, the data do suggest a progressive pattern to the ABR changes associated with bilirubin administration. Initially, there appear to be variable wave latency and amplitude changes unassociated with clinical symptoms. These changes are followed, in susceptible animals, by ABR wave loss and the onset of clinical symptoms of bilirubin toxicity.

It is possible that the ABR amplitude and latency changes observed are related to a readily reversible bilirubin "effect" or toxicity in the chochlea or auditory nerve, while ABR wave loss reflects a progression of these effects and/or a more global and

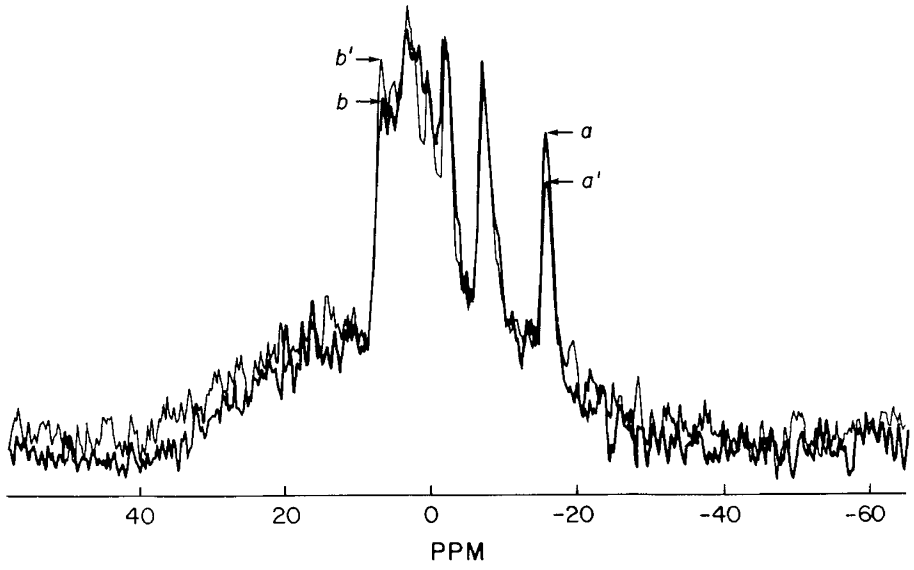

Fig. 4. NMR changes in a 1-day-old term infant after bilirubin and sulfisoxazole. The ATP peak fell from a to a′ with a rise in the inorganic phosphate peak from b to b′.

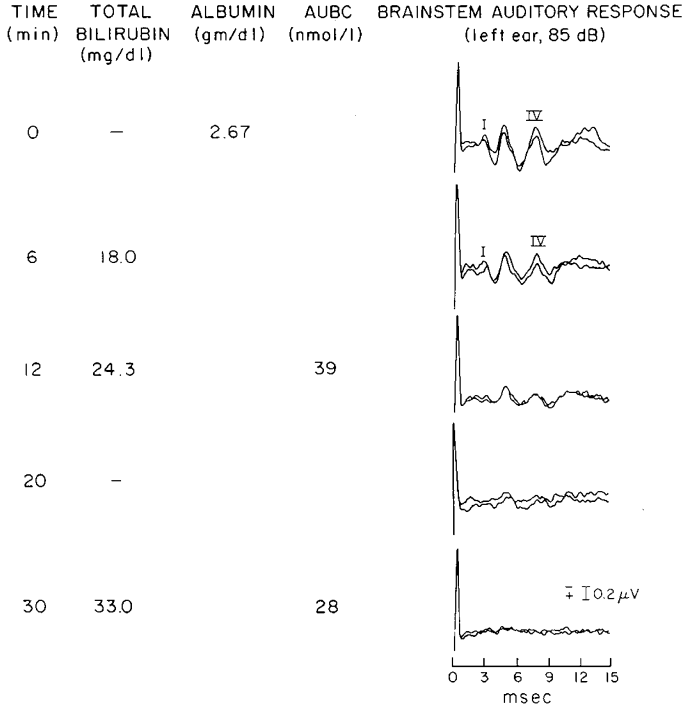

Fig. 5. Changes in ABR associated with administration of 90 mg/kg of bilirubin in a premature rhesus infant. Total bilirubin is the serum bilirubin concentration. AUBC is the apparent unbound bilirubin concentration. Ten nmol/liter of unbound bilirubin is about 0.6 μg/dl.

serious toxic effect on the central nervous system as well. We do have some evidence in support of a global toxic effect as P_{31} nuclear magnetic resonance spectroscopy, somatosensory evoked potentials, and the electroencephalogram were also monitored in the 1-day-old term infant. The animal had shown no change in any of the monitoring patterns until sulfisoxazole was given. The loss of ABR was associated with a quickly reversed, rapid fall in brain ATP and rise in inorganic phosphate on the P_{31} nuclear magnetic resonance spectrum, a loss and recovery of the electroencephalogram, and a conduction block of the somatosensory evoked potential in the posterior column-lemniscal system at or rostral to the brainstem. These findings suggest significant, transient dysfunction throughout the central nervous system with bilirubin/sulfisoxazole-induced ABR loss.

It has been unclear whether preexisting factors such as asphyxia are necessary for bilirubin toxicity to occur (11, 15, 18). Preterm human infants appear to be susceptile to direct bilirubin toxicity with no confounding factors other than prematurity (21). Our preterm infants in whom we prevented asphyxia, acidemia, hypoglycemia, and hypothermia (see "Methods") appeared very susceptible to bilirubin alone. The data suggest a direct ototoxic bilirubin effect in the premature rhesus infant.

This model may be useful in clarifying many of these issues and improving clinical care of the jaundiced infant. The major potential limiting factor is animal cost. However, the ease of working with these animals as well as the large body of data on their neurological and social development would justify the cost. There is dissimilarity in the wave generators in the monkey and human, and whether this would have a major impact on the applicability of the monkey data to humans is unclear. This issue can only be assessed by further animal and human studies which will more clearly define the anatomical locations of the toxic bilirubin effects.

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Announcement

An NIH Consensus Development Conference on Infantile Apnea and Home Monitoring

The conference will be held September 29 and 30 and October 1, 1986 at the Masur Auditorium, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD.

It is sponsored by the National Institute of Child Health and Human Development; the National Heart, Lung, and Blood Institute; the Division of Maternal and Child Health, HRSA; the Food and Drug Administration; and the NIH Office of Medical Applications of Research.

Discussion will center on the specific indications for the use of home monitoring and for its discontinuation. A draft report, including options for a consensus statement, will be prepared by the consensus development panel and distributed to all registrants prior to the meeting. The draft report will consider the following key questions: 1) What is known about the relation of neonatal and infant apnea to each other and to mortality (especially SIDS) and morbidity in infancy? *2*) What is the efficacy and safety of currently available home devices for detecting infant apnea? *3*) What evidence exists regarding the effectiveness of home monitoring in reducing infant mortality (especially SIDS) and morbidity? *4*) What recommendations can be made at present regarding the circumstances for the use of home apnea monitoring in infancy? *5*) What further research is needed on home apnea monitoring for infants?

The conference will bring together pediatricians, neonatologists, family practitioners, epidemiologists, medical engineers, nurses, parents, and members of the public. Following 2 days of presentations by experts and members of the panel and comments by individuals and organizations in addition to discussion from the audience, the panel will consider the available evidence and modify its draft report as needed.

To register to attend the conference, contact Ms. Barbara McChesney, Prospect Associates, Suite 401, 2115 East Jefferson Street, Rockville, MD 20852, (301) 468-655.