Navajo jaundice: A variant of neonatal hyperbilirubinemia associated with breast feeding

A clinical and chemical appraisal of 47 newborn Navajo infants demonstrated a high incidence of unconjugated hyperbilirubinemia during the first week of life. Within this study population two subgroups were identified: (1) breast-fed infants in whom inhibition of bilirubin glucuronyl transferase activity was related to a substance in colostrum and breast milk in the first days of life and (2) a smaller subgroup of bottle-fed infants who had significant jaundice when compared with a control population from New York. The serum bilirubin concentrations of the Navajo bottle-fed infants never achieved those of the breast-fed subgroup. These data suggest that to some extent the neonatal unconjugated hyperbilirubinemia noted in the Navajo Indian is related to breast feeding, with earlier transmission of the presumed inhibitor substance than previously observed in a Caucasian population.

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AN UNUSUAL pattern of neonatal unconjugated hyperbilirubinemia appearing in the first six days of life among American Indians has been suggested previously.[1, 2] The etiology of this syndrome remains obscure. The occurrence of significantly higher levels of unconjugated hyperbilirubinemia during the first week of life among neonates of other Mongolian groups as opposed to Caucasian newborn infants has been described.[3] The phenomenon of delayed development of the bilirubin glucuronyl transferase enzyme system has often been employed to explain this early and transient hyperbilirubinemia in both racial groups.[1, 4, 5] The clinical observation that many Navajo Indian newborn infants are jaundiced and simultaneously breast fed suggested to us the possibility that a breast-milk-transmitted inhibitor of bilirubin glucuronyl transferase[6] may in part be involved in the pathogenesis of this syndrome, although the very early onset of icterus was distinctly different from the reported experience with breast-milk jaundice in most Caucasian infants.[7]

Abbreviations used	
UDP:	uridine diphosphate
GA:	glucuronic acid

The present study describes further clinical aspects of this syndrome of unconjugated hyperbilirubinemia in Navajo infants and suggests a partial explanation as to the etiology.

MATERIALS AND METHODS

Forty-seven infants born consecutively at the United States Public Health Service Indian Hospital in Tuba City, Ariz., during a ten-week period were included in the study. All babies were considered full-blooded Navajo Indians except for four whose lineage was partially from the Hopi tribe. The birth weights in all were greater than 2,500 grams. Sepsis, hemolytic disease, and other

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conditions associated with hyperbilirubinemia were excluded. The only medication administered to all mothers during pregnancy was vitamins. A vitamin K_1 analogue was given just after delivery to all infants. Two of the 47 deliveries were abnormal, requiring cesarean section for cephalopelvic disproportion. None of the mothers was clinically icteric. Thirty-nine infants were breast fed, and eight were offered a commercially available milk formula. The method of feeding was entirely a maternal decision.

Cord blood was obtained from 43 of the 47 infants and 93 heel-stick specimens from the 47 babies were subsequently obtained from one to six days of age. All specimens were immediately centrifuged, wrapped in foil, and stored at −20° C for analysis of bilirubin concentrations and for the presence of a circulating inhibitor of bilirubin glucuronyl transferase. Bilirubin concentrations were determined[8] at the USPHS Indian Hospital, while paired samples were centrifuged, wrapped in foil, and frozen for similar analysis at Montefiore Hospital.

Similar blood collection and preservation was carried out for 25 of the 47 Indian mothers on the second or third postpartum day.

Breast milk or colostrum was obtained manually or by breast pump from 27 of the 39 breast-feeding mothers and from three of the eight non-breast-feeding mothers from postpartum days 0 through 6. Lastly, maternal urine specimens were collected from 15 breast-feeding and five non-breast-feeding subjects. These urine and milk samples were stored at −20° C. All maternal serum, urine, and breast-milk specimens were studied for their possible inhibitory effect on bilirubin glucuronide formation in rat homogenates.

At the completion of the ten-week study period, specimens were coded and mailed by air freight to Montefiore Hospital. All specimens arrived in the frozen state.

A control group of 36 healthy, term, breast- and bottle-feeding infants born to black, Hispanic (Puerto Rican), or white mothers at the Morrisania City Hospital, New York City, were studied simultaneously to determine the mean serum bilirubin concentration and the incidence of inhibitors of glucuronyl transferase in breast milk in a non-Indian neonatal population. One hundred one heel-stick specimens from these 36 babies were obtained during the first four days of life.

Breast milk was collected from 17 black, Hispanic, and white mothers in the control group during the first postpartum week. This milk was frozen and stored in a manner identical to the Navajo specimens and for the same length of time. Serum bilirubin was determined by the method of Malloy and Evelyn.[8] Bilirubin uridine diphosphate (UDP) glucuronyl transferase (EC 2.4.1.17) was estimated by the method cited by Black and Billing[9] and a modification of the method of DeLeon and associates[10] and Gartner.[11] This modification included the addition of 2% digitonin in 0.154M KCl added to an 8% liver homogenate in a 1:1 dilution just prior to the addition of 0.1 ml of UDP glucuronic acid (GA) (6.6×10^{-2}).

The inhibition studies utilized maternal, cord, or neonatal sera, or maternal urine, added as potential inhibitor-containing solutions to the reaction mixture in either 0.2 or 0.4 ml concentration to a total volume of 1.7 ml. Breast milk was added in 0.1 and 0.2 ml aliquots to the same total volume. The inhibition study, outlined previously by Arias and associates,[6] utilized adult male Sprague-Dawley rat liver as the enzyme source with added UDPGA.

RESULTS

Clinical course. Of the 47 Navajo infants, 45 had Apgar scores determined at one minute and 39 had a score of 8 or higher. Only one infant with a score of 5 required brief pulmonary resuscitation. None of the study group infants died and no signs of kernicteric encephalopathy were observed. There is no evidence that significant hyperbilirubinemia in the American Indian tribes noted by others[1,2] ever produced clinical kernicterus.

Four patients were treated with phototherapy, two on day 3 and two on day 4 of life. All were breast-fed and, although clinically well and without evidence of isoimmunization or sepsis, had serum bilirubin values at the initiation of phototherapy as follows: 18.2 and 20.0 mg/100 ml on day 3, and 17.4 and 23.6 mg/100 ml on day 4, respectively.

No infant required exchange transfusion and all 47 babies were discharged home in good health between 3 and 6 days of age but with clinical icterus still present in most. The 36 infants in the control group in New York City had an uneventful neonatal course.

The mean birth weight of the 47 Navajo babies was 3,453 grams (S.E. 60) with a range of 2,637 to 4,366 grams. The control population had a mean birth weight of 3,177 grams (S.E. 71) with a range of 2,510 to 3,856 grams.

Hematologic studies. No evidence of isoimmunization was found in 40 of 47 Navajo infants studied. The control population was also free of isoimmunization problems. Results of sequential hematocrit determinations in the Navajo babies revealed no significant fall over the week-long period of observation.

Serum bilirubin concentrations were determined in 43 cord blood and 120 capillary and peripheral blood specimens from the Navajo group and in 101 capillary and peripheral blood samples from the control group. A signifi-

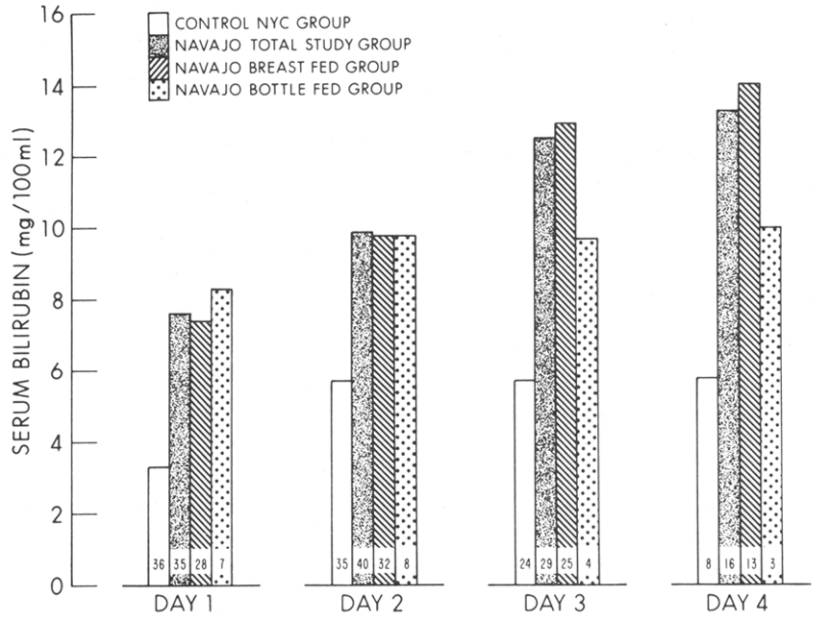

Fig. 1. Serum bilirubin concentrations during the first 4 days of life. A control group of infants born in New York City is depicted by the clear bars while the total Navajo study group is represented by the dark bars. Subgroups of breast-fed and bottle-fed Navajo babies are shown as striped and stippled areas, respectively. The height of each bar depicts the mean total daily bilirubin concentration. The digits within each bar represent the number of infants sampled on that day. Statistically signficant differences between the control group and the total Navajo group exist on each of the 4 days ($p < 0.001$). Within the Navajo subgroup, differences exist only on days 3 and 4 but statistical analysis could not be determined because of the limited number of samples during these two periods.

cant difference ($p < 0.001$) between the control and study populations on all four days for which a significant number of specimens was available (Fig. 1) was found. Within the Navajo group, both breast-fed and non–breast-fed babies showed a very similar hyperbilirubinemia through the second day of life. On days 3 and 4, however, the breast-fed population continued to show rising concentrations of bilirubin whereas in the bottle-fed group bilirubin concentrations appeared to plateau at the 10 mg/100 ml range. No data were available after day 4 in the majority of infants. The direct-reacting bilirubin fraction never comprised more than 8% of the total pigment assayed.

Inhibition studies. The mean per cent inhibition by milk of bilirubin glucuronyl transferase activity in rat liver homogenates from 27 of the 39 breast-feeding Navajo mothers is presented in Table I. Three milk specimens from non–breast-feeding Navajo mothers are also shown (specimens 28 to 30). Seventeen milk specimens from the maternal New York City control group had degrees of enzyme inhibition ranging from 0 to 16%. A 20% or more degree of in vitro inhibition of formation of bilirubin glucuronide was considered abnormal. This value compares favorably with previously published reports.[6, 7] The mean per cent inhibition for all Navajo breast milk was 29.9 while that for the control group was 3.8%. These data are statistically significant with the use of Student's t test ($p < 0.001$).

Of the 27 assayed Navajo milk specimens from breast-feeding mothers, all but five (specimens 1, 3, 5, 8, 13) displayed a significant degree of inhibition of the transferase system. It is noteworthy that the higher the bilirubin concentration in any individual baby the more inhibitory activity we appreciated in that baby's maternal breast milk.

Although there was good correlation between Navajo breast-feeding practices, unconjugated hyperbilirubinemia, and the presence of breast-milk inhibition of bilirubin glucuronyl transferase activity, an appreciable degree of unexplained neonatal jaundice still existed in our non–breast-fed Indian study population. Fig. 2 shows the results of a series of other enzymatic assays performed in an attempt to identify a circulating inhibitor of bilirubin glucuronyl transferase in maternal, cord, or newborn infant sera from this Navajo subgroup. Maternal urine was similarly assayed for evidence of a possible inhibitor substance. No inhibitory association could be found in any of the three sera sources or maternal urine. Indeed, the mean enzyme activity appeared to approach zero with regard to enhancement vs. inhibition of enzyme activity upon addition of either serum or urine in a significant number of the 95 duplicate assays

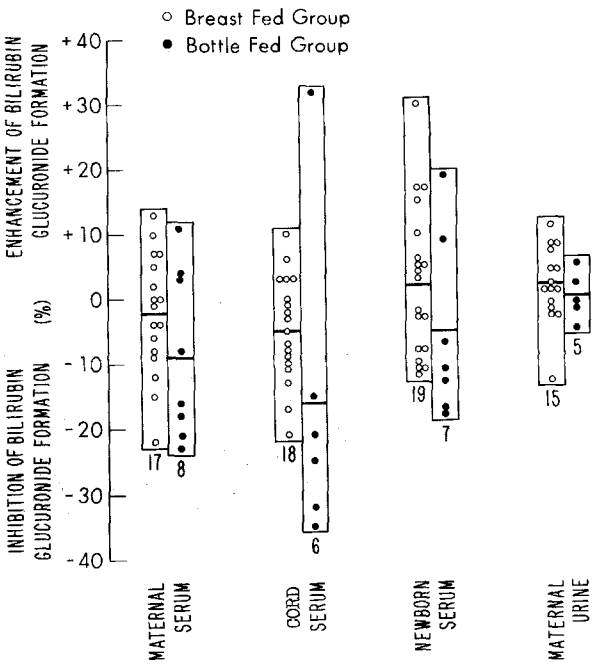

Fig. 2. The percentages of enhancement or inhibition of bilirubin glucuronide formation by rat liver homogenates after the addition of potential inhibitory solutions from the Navajo study groups are demonstrated along the ordinate. Each circle represents a single specimen: the open circles from breast-feeding maternal-child pairs: the dark circles from bottle-feeding maternal-child pairs. The heavy horizontal lines represent the mean values for each group and none are noted to be at 20% inhibition or greater.

performed. Four cord specimens from anicteric non-breast-fed babies did show inhibition greater than 20% but serum specimens obtained from one to four days later failed to replicate the trend.

Large quantities of Navajo breast milk could not be collected during the brief hospitalization or once the maternal-infant pair had been returned home so that the identification of the potential inhibitor substance in the milks was not possible. The causative agent associated with breast feeding in white infants has been identified as pregnane-3-(alpha), 20-beta-diol by several investigators[6,12] but others[13,14] have recently questioned the specific inhibitory effect of this steroid. During our enzyme assay studies, we attempted to control for inhibition and lack of it by utilizing varying concentrations of both pregane-3(alpha),20-beta-diol and pregnane-3-(alpha), 20-alpha-diol dissolved in 70 to 100% alcohol (Sigma Chemical Co., St. Louis, Mo.). Concentrations of both steroids varying from 0.06 to 114 μg/ml were utilized in both rat liver homogenate and rat liver slice preparations[6] but failed to show any inhibitory effect by either steroid on the formation of bilirubin glucuronide in the presence of adequate concentrations of UDPGA.

Table I. Navajo maternal breast milk inhibition of rat bilirubin glucuronyl transferase activity in vitro showing the postpartum day that the milk was obtained, the per cent inhibition in each milk sample, and the total infant serum bilirubin level achieved on that day. Specimens 1 to 27 were from mothers who breast fed; specimens 28 to 30 came from mothers who bottle fed their infants

Specimens (No./day)	*Per cent inhibition*	*Total bilirubin*
1/1	19	7.8
2/1	37	11.9
3/2	11	3.1
4/2	23	10.3
5/2	14	9.2
6/2	20	10.0
7/2	26	10.2
8/2	18	9.9
9/2	21	12.8
10/2	23	11.0
11/2	22	8.6
12/2	90	12.6
13/2	10	8.5
14/2	22	8.1
15/2	20	12.5
16/2	38	11.4
17/3	40	15.0
18/3	20	8.0
19/3	85	11.2
20/3	67	14.1
21/3	27	13.0
22/3	26	10.0
23/3	29	11.0
24/3	66	14.6
25/3	23	11.5
26/5	47	13.6
27/8	25	13.3
28/2	3	4.0
29/2	4	8.1
30/3	20	9.2

DISCUSSION

Several recent studies have reported significant degrees of hepatobiliary disease among adult American Indians,[15-17] including the Navajo,[18] but there has been only minimal emphasis and study of neonatal jaundice among these peoples. In 1966 Trygstad and McCracken[1] described physiologic jaundice in a group of Sioux, and Chopra and associates[2] commented on significant unconjugated hyperbilirubinemia among Southwestern American Indian neonates, although the tribes involved were not specified. No complete explanation for this jaundice is currently at hand.

The clinical and laboratory data reported herein suggest this phenomenon is at least in part related to an inhibitor of bilirubin glucuronyl transferase transmitted in breast milk. Placental transfer of inhibitor substance as reported by Lucey[19] and Arias[20] and their colleagues but utilizing ortho-aminophenol as the glucuronide acceptor rather than bilirubin was excluded by our assays of maternal, cord, and neonatal serum. Prematurity, sepsis, respiratory distress, hemolytic disease, and icterogenic-producing drugs were similarly excluded as etiologic factors in the hyperbilirubinemia of these infants.

As in other studies describing jaundice associated with breast milk, no evidence of kernicteric episodes were noted. Long-term follow-up with neuropsychologic appraisal is needed to be certain more subtle signs are not being obscured.

In all Navajo infants displaying high serum bilirubin concentrations early in the first week of life, the inhibitory activity of the breast milk was present within the first 72 hours. This observation is in marked contrast to the more traditional breast milk jaundice syndromes occurring at two weeks of age and described in white infants.[7] Indeed, several of the specimens obtained and assayed were colostrum and these also displayed inhibitory activity, although the inhibition seemed to be maximal at about 72 hours.

The finding of significant inhibition of bilirubin glucuronide formation in most of the specimens of Navajo breast milk that were assayed, notwithstanding, one still has the difficult task of relating the elevated serum bilirubin values in non-breast-fed Navajo neonates compared to our control population in New York City. We would suggest that still other, as yet unexplained, genetic and/or environmental factors probably play a role. Climate, maternal diet, and the general environment are certainly different, consanguineous marriage among Indian tribal groups is frequent, and a higher incidence of hepatobiliary disease among American Indians has already been cited. Many of these factors may play a significant role in the etiology of neonatal jaundice in the Navajo.

The data from this laboratory, coupled with similar reports from Great Britain,[13, 14] suggesting that the 20-beta isomer of pregnandiol may not be inhibitory to bilirubin glucuronyl transferase, further complicate the issue. Clarification of this point remains essential to understanding the pathophysiology of breast milk jaundice.

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