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Research Article

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Transient Familial Neonatal Hyperbilirubinemia *

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Normal full-term infants may have transient unconjugated hyperbilirubinemia that rarely exceeds 5 mg per 100 ml during the first 3 to 5 days of life (1, 2). This so-called physiologic hyperbilirubinemia is believed to result from delayed development of the hepatic glucuronide conjugating system (3–5), particularly glucuronyl transferase (6). Numerous factors, such as hemolysis, infection, drugs, and prematurity influence the severity of unconjugated hyperbilirubinemia in newborn infants; however, many cases of severe neonatal unconjugated hyperbilirubinemia are observed for which no etiologic explanation is available (7, 8). The possibility that some of these cases may have a familial basis has not been previously suggested.

Our attention was called to this possibility by observations made during the last 7 years on five mothers who had given birth to a total of 16 infants each of whom had severe transient unconjugated hyperbilirubinemia for which no adequate explanation was found. Three of these infants died of kernicterus, and one surviving child had quadriplegic cerebral palsy that probably resulted from kernicterus. Subsequent to preliminary reports of this syndrome (9, 10), three additional families became available for study.

In 1957 Lathe and Walker demonstrated that serum from pregnant women and their newborn infants regularly inhibits the formation of conjugated bilirubin by rat liver slices *in vitro* (11). In 1958 we suggested that transient familial neonatal hyperbilirubinemia may result from increased amounts of a substance in the serum of certain pregnant women that inhibits the hepatic conjugation of bilirubin (9, 10). Studies of serum inhibitor factor activity were, therefore, performed during subsequent pregnancies in some of these patients.

The results indicate that, beginning in the second trimester of pregnancy, mothers of infants with transient familial neonatal hyperbilirubinemia have significantly greater serum inhibitor factor activity than do normal pregnant women. This observation is probably important in the etiology and pathogenesis of transient familial neonatal hyperbilirubinemia.

Methods

Data regarding the health of the eight mothers before, during, and after each pregnancy were obtained from hospital charts and records of private physicians. A family history of severe or prolonged neonatal jaundice was specifically sought. Each of the 24 infants was born in a hospital, and data regarding birth and the neotal period were obtained from hospital records.

The following studies were performed by standard techniques to determine the cause of severe neonatal jaundice: *1*) blood typing and Rh factor determination, Coombs' test, examination of peripheral blood morphology, estimation of hemoglobin concentration, and reticulocyte count; *2*) serologic test for syphilis; and *3*) blood cultures. Serum direct-reacting and indirect-reacting bilirubin concentrations were estimated according to Malloy and Evelyn (12). Erythrocyte glucose-6-phosphate dehydrogenase activity was estimated in three infants, urinary sediment was examined for evidence of cytomegalic inclusion body disease in four infants, and serum glutamic-oxaloacetic and glutamic-pyruvate transaminase activities and cephalin-cholesterol flocculation were estimated in three infants.

The following specimens of serum were obtained, frozen, and stored at − 4° C until assayed for inhibitor factor activity:

a) Two specimens were obtained from each of 20 nonpregnant female laboratory personnel of child-bearing age.

b) Eighty-four specimens were obtained from 16 pregnant women whose previous infants had not been severely jaundiced. These sera were obtained during each trimester, at term, and during the first 2 weeks postpartum,

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and with the sera from nonpregnant women these specimens served as controls.

c) Fifty-eight specimens were obtained from the eight mothers of infants with transient familial neonatal hyperbilirubinemia during and after 11 pregnancies.

d) Thirty specimens were obtained from 15 normal infants during the first 14 days of life.

e) Twelve specimens were obtained from 11 infants with transient familial neonatal hyperbilirubinemia during the first 3 months of life.

Male Wistar rats weighing 100 to 150 g were fed mouse breeder chow and water ad libitum until sacrificed by decapitation. The liver was quickly removed, chilled, and washed in 0.154 M KCl. Liver slices were prepared with a Stadie blade. Fifty to 75 mg wet weight of liver slices was placed in each incubation flask. Ten per cent liver homogenates were prepared in 0.154 M KCl with a motor-driven Teflon pestle and a glass homogenizer. All operations were performed at 0° C.

Serum was thawed to room temperature. The effect of serum on direct-reacting bilirubin formation by rat liver slices was estimated in quadruplicate according to the method of Lathe and Walker (11). The effect of a final serum concentration of 15% on *o*-aminophenol glucuronide formation by homogenates of rat liver was studied according to the method of Hsia, Dowben, Shaw, and Grossman (13). The incubation mixture consisted of 0.5 M Tris buffer (pH 7.8), 0.2 ml; 0.5 M $MgCl_2$, 0.2 ml; 1.1×10^{-3} M uridine diphosphate glucuronic acid (UDPGA), 0.2 ml; 1.25×10^{-3} M *o*-aminophenol in 0.02 M ascorbic acid, 0.4 ml; 10% rat liver homogenate, 2.0 ml; and serum, 0.44 ml. Incubation was in duplicate at 37° C for 30 minutes. *o*-Aminophenol glucuronide formation was estimated according to Levvy and Storey (14). The mean estimates of direct-reacting bilirubin and *o*-aminophenol glucuronide formation in the presence of test sera were compared to those observed with control male serum, and the result was expressed in arbitrarily defined units of inhibition. One unit is equivalent to 1% inhibition produced by undiluted serum in the test system. When a test serum produced inhibition exceeding 90%, it was serially diluted with control male serum, and the assay was repeated. The control male serum had negligible inhibitory activity *in vitro*. Serum with increased inhibitory activity showed proportional decreases in inhibitory activity as it was diluted increasingly with normal serum.

Five specimens of inhibitory serum were studied further. Their effect on direct-reacting bilirubin and *o* aminophenol glucuronide formation by rat liver slices and homogenates, respectively, was studied after the sera were *1*) stored at −10° C for 6 months; *2*) immersed in a boiling water bath for 1 hour, centrifuged, and the supernate examined, and *3*) dialyzed at 3° C for 2 days against flowing isotonic saline.

Results

A) *Clinical results.* Clinical data concerning the eight mothers and their 24 infants are summarized in Tables I and II. Further details regarding each infant are presented in the Appendix.

TABLE I

Transient familial neonatal hyperbilirubinemia: clinical observations

Family	Blood type		Sex	Birth weight	Gestational age	Highest serum bilirubin	Day observed	No. of exchange transfusions
	Mother	Baby		*g*	*weeks*	*mg/100 ml*		
G.O.	O+	O+	Female	2,792	34	48	5	
		O+	Female	3,090	40	36	5	1
V.H.	A+	O+	Male	2,948	36	Deeply jaundiced	4	
		A+	Male	2,977	40	61	7	2
		O+	Female	3,050	40	25	3	2
		O+	Male	3,345	40	17	5	0
A.C.	O+	O+	Male	1,760	31	20	5	2
		O+	Male	3,444	40	12.6	4	0
		O+	Female	2,892	39	8.9	4	0
D.F.	B+	O+	Male	2,126	34	65	5	2
		B+	Female	1,913	34	40	5	1
		O+	Female	3,416	41	22	3	2
		B+	Female	3,657	40	13	3	0
		O+	Male	4,224	41	15	3	0
H.S.	O+	A−	Female	1,559	34	31	5	0
		A−	Female	2,537	34	18	4	0
W.G.	A+	O+	Male	3,000	32	18	36 hours	0
		A+	Female	3,200	35	14	4	0
		A+	Male	3,400	40	24	3	0
		A+	Male	3,910	40	19	4	0
N.R.	O+	O+	Male	3,600	40	19	4	0
		O+	Male	3,700	40	16	5	0
J.F.	B+	O+	Female	3,500	36	23	5	0
		B+	Male	3,700	40	17	3	0

TABLE II

Transient familial neonatal hyperbilirubinemia: clinical observations

Family	Age at death	Post-mortem	Present age	Follow-up
			years	
G.O.			7	Normal
			5	Normal
V.H.			8	Normal
	11 months	Kernicterus		
			4	Normal
			3	Normal
A.C.			2	Cerebral palsy
			4	Normal
			3	Normal
D.F.	6 days	Kernicterus		
			7	Normal
			2	Normal
			5	Normal
			4	Normal
H.S.	6 days	Kernicterus		
			5	Normal
W.G.	36 hours	Atalectasis		
			8	Normal
			5	Normal
			2	Normal
N.R.			4	Normal
			1	Normal
J.F.			4	Normal
			3	Normal

The eight mothers were Caucasians and had always been healthy. A.C. and D.F. are sisters-in-law, but otherwise the eight mothers were unrelated to each other. There was no history of diabetes, chronic jaundice, abnormal menstruation, or steroid ingestion during pregnancy. Unexplained and severe neonatal jaundice had not been observed in the eight mothers or in their siblings or parents.

In each of the 24 infants jaundice was noted within the first 4 days of life. The maximal observed serum bilirubin concentrations ranged from 8.9 to 65 mg per 100 ml (mean, 25.4 mg per 100 ml) and were detected on the third to seventh days of life. The serum concentration of direct-reacting bilirubin never exceeded 5% of the total serum bilirubin concentration.

Laboratory studies failed to reveal any known etiology for the severe neonatal unconjugated hyperbilirubinemia. The possibility of a major blood group incompatibility existed only in family H.S., but was excluded by failure to demonstrate hyperimmune levels of anti-A antibodies in the cord blood. Coombs' tests were negative in each of the 24 infants. There was no significant anemia in the 18 infants in whom hemoglobin concentrations were determined. Reticulocyte counts were within the range of normal encountered at this age. Peripheral blood smears in 18 infants did not reveal spherocytosis or erythroblastosis. Erythrocyte glucose-6-phosphate dehydrogenase activity was normal in the three infants on whom this study was performed. Pyknocytes were searched for in 11 cases, but none were seen. Six infants (V.H. No. 1, 2, 3, and 4; D.F. No. 3; and W. G. No. 1) were breast-fed.

Each mother had a negative serologic test for syphilis. Blood cultures, which were obtained in ten infants, were negative. Four infants had negative studies of urinary sediment for evidence of cytomegalic inclusion body disease. Petechiae, splenomegaly, or other signs of infection were not noted in any of the infants.

Except for A.C., who received Kynex, a long-acting sulfonamide, for 7 days before delivery of baby A.C. No. 1, no medications known to increase the severity or duration of neonatal hyperbilirubinemia were taken by the eight mothers during pregnancy. Four infants (G.O. No. 1, D.F. No. 1, D.F. No. 2, and H.S. No. 1) received a total of 12.5 mg of Hykinone in divided doses during the first 5 days of life. The other infants received 2.5 mg of Hykinone intramuscularly at birth and 2.5 mg on the third day of life. Five infants (G.O.

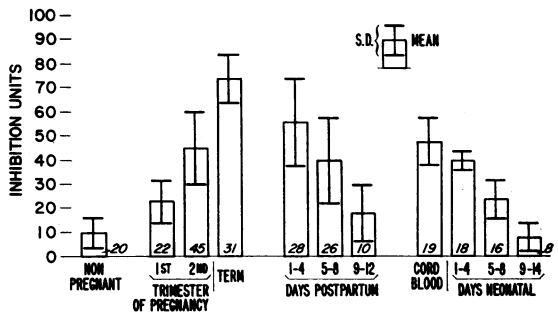

FIG. 1. INHIBITION OF DIRECT-REACTING BILIRUBIN FORMATION BY RAT LIVER SLICES BY SERUM FROM NORMAL NONPREGNANT, PREGNANT, AND POSTPARTUM WOMEN AND THEIR NEWBORN INFANTS. The numbers in each bar indicate the number of sera studied.

No. 1, D.F. No. 1, D. F. No. 2, D. F. No. 5, and J.F. No. 1) received penicillin and streptomycin subsequent to exchange transfusion.

Serum glutamic-oxaloacetic and glutamic-pyruvic transaminase activities and cephalin-cholesterol flocculation were normal in the three infants in whom these studies were performed. Histologic examination of the liver at autopsy was normal in four infants (V.H. No. 2, D.F. No. 1, H.S. No. 1, and W.G. No. 1).

None of the 20 surviving children had jaundice that persisted after the neonatal period, nor was there a history of jaundice in other family members.

Three infants had birth histories suggesting possible intrauterine anoxia. One infant (W.G. No. 1) died 36 hours after birth from respiratory distress and atelectasis.

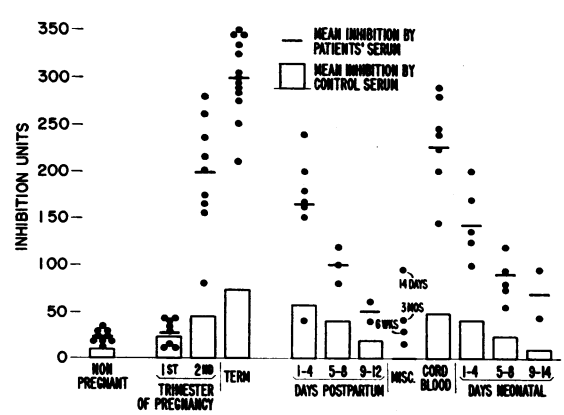

FIG. 2. INHIBITION OF DIRECT-REACTING BILIRUBIN FORMATION BY RAT LIVER SLICES BY SERUM FROM INFANTS WITH TRANSIENT FAMILIAL NEONATAL HYPERBILIRUBINEMIA AND THEIR PREGNANT MOTHERS.

Four infants weighed less than 2,500 g and were, therefore, considered premature.

B) Laboratory results. The mean inhibition of direct-reacting bilirubin formation by rat liver slices by sera from control nonpregnant women, normal women during and after pregnancy, and from normal newborn infants is presented in Figures 1 and 2. The results are similar to those published by Lathe and Walker (11). Quadruplicate estimations of direct-reacting bilirubin formation by rat liver slices with and without inhibitory serum did not exceed the mean by more than 22%. Serum inhibitory activity increased during pregnancy, was less in cord blood than in maternal serum at term, and became normal in postpartum and neonatal serum by the tenth day after delivery.

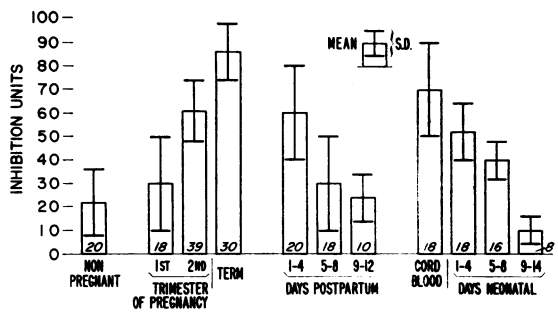

FIG. 3. INHIBITION OF O-AMINOPHENOL GLUCURONIDE FORMATION BY RAT LIVER HOMOGENATES BY SERUM FROM NORMAL NONPREGNANT, PREGNANT, AND POSTPARTUM WOMEN AND THEIR NEWBORN INFANTS. The numbers in each bar indicate the number of sera studied.

The mean inhibition of *o*-aminophenol glucuronide formation by rat liver homogenates by sera from control nonpregnant women and from normal women during and after pregnancy and from normal newborn infants is presented in Figures 3 and 4. The results are similar to those published by Hsia and associates (13). Duplicate estimations of *o*-aminophenol glucuronide formation by rat liver homogenates with and without inhibition serum were within 14% of each other. The results follow a pattern similar to the inhibition of direct-reacting bilirubin formation by rat liver slices shown in Figure 1.

Figure 2 presents the mean inhibition of direct-reacting bilirubin formation by rat liver slices by serum obtained from the eight mothers of infants with transient familial neonatal hyperbilirubinemia

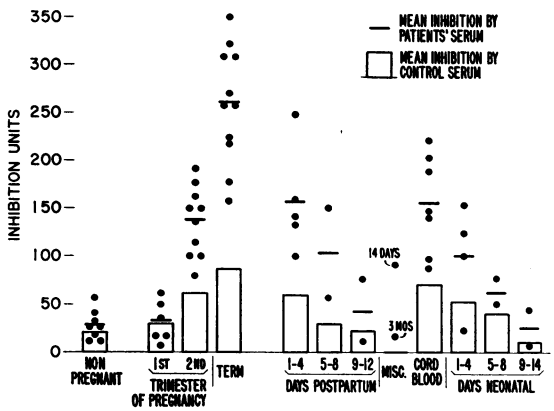

FIG. 4. INHIBITION OF O-AMINOPHENOL GLUCURONIDE FORMATION BY RAT LIVER HOMOGENATES BY SERUM FROM INFANTS WITH TRANSIENT FAMILIAL NEONATAL HYPERBILIRUBINEMIA AND THEIR PREGNANT MOTHERS.

and 11 of their infants. As indicated in Figure 2, maternal sera obtained before pregnancy and during the first trimester of pregnancy were undiluted when studied. All sera subsequently obtained were diluted 1:4 to 1:10 with noninhibitory, normal male serum. Beginning in the second trimester of pregnancy, the inhibitory activity of serum from mothers of infants with transient familial neonatal hyperbilirubinemia was four to ten times greater than that observed with maternal serum in the control group.

Figure 4 presents the mean inhibition of *o*-aminophenol glucuronide formation by rat liver homogenates by serum obtained from the eight mothers of infants with transient familial neonatal hyperbilirubinemia and eight of their infants. Maternal serum from the second trimester of pregnancy through term was diluted 1:2 as previously described. Serum from the neonates was undiluted when studied. The temporal course of serum inhibitory activity during and after pregnancy and in the neonatal period is similar to that observed in Figure 2. The degree of inhibition is less in each subsequent time period after birth. The inhibitory activity became normal by the fourteenth day in both postpartum and neonatal serum, but remained elevated for 3 months in one mother (H.S.) and for 2 weeks in one infant (J.F. No. 2).

The effect of five inhibitory sera on direct-reacting bilirubin and *o*-aminophenol glucuronide formation by rat liver slices and homogenates, respectively, was not altered by *1*) storage of serum at − 10° C for 6 months, *2*) immersion of serum in a boiling water bath for 1 hour, or *3*) dialysis at 3° C for 2 days against flowing isotonic saline.

Discussion

To establish that transient familial neonatal hyperbilirubinemia is a nosologic entity, one must exclude factors known to be etiologically related to neonatal unconjugated hyperbilirubinemia. The available data on the 24 infants do not implicate hemolysis, infection, drug effect, maternal disease, liver damage, respiratory distress syndrome, prematurity, or breast feeding as causing the hyperbilirubinemia. There is no known inherited defect in bilirubin metabolism that results in transient unconjugated hyperbilirubinemia. An inherited defect of glucuronyl transferase activity results in permanent unconjugated hyperbilirubinemia (15–17).

Clinically transient familial neonatal hyperbilirubinemia differs from the syndrome of transient nonhemolytic unconjugated hyperbilirubinemia associated with breast feeding in some infants (18–20). In the latter syndrome, severe and prolonged unconjugated hyperbilirubinemia is observed in breast-fed but not bottle-fed infants of mothers whose breast milk contains pregnane-3(α),20(β)-diol that competitively inhibits hepatic glucuronyl transferase activity *in vitro* (19). Serum obtained from these mothers does not have significantly greater inhibitor factor than normal pregnancy serum. Kernicterus has not been observed in infants with jaundice associated with breast feeding, possibly because severe jaundice does not occur until the seventh to tenth day of life at which time the blood-brain barrier is presumably relatively impermeable to unconjugated bilirubin (8). Only 6 of the present 24 infants were breast-fed.

Lathe and Walker described the inhibitory effect of normal pregnancy serum on direct-reacting bilirubin formation by slices of rat liver and postulated that the inhibitor was a progestational steroid (11). Hsia and co-workers isolated pregnane-3(α),20(α)-diol as the major inhibitor in pooled pregnancy serum and demonstrated that the steroid competitively inhibits glucuronyl transferase activity in hepatic microsomes (13, 21). Holton and Lathe have claimed that pregnane-3(α),20(α)-diol does not inhibit glucuronide formation by

slices of human liver and suggested that an unidentified steroid may be the active inhibitor in man (22). Equimolar amounts of pregnane-3(α),-20(α)-diol and pregnane-3(α),20(β)-diol equally and competitively inhibit glucuronyl transferase activity in guinea pig liver microsomes *in vitro* (19). Administration of the latter isomer of pregnanediol to normal full-term infants resulted in nonhemolytic unconjugated hyperbilirubinemia that disappeared when steroid administration was discontinued (20). Serum from mothers of infants with transient familial neonatal hyperbilirubinemia presumably contains the normally occurring inhibitors associated with pregnancy. Whether the increased inhibitory effects of these sera result from qualitative or quantitative differences is unknown.

The inhibitor in the present study is nondialyzable, heat and cold stable, and temporarily associated with pregnancy. These observations suggest that it is probably a progestational steroid. Attempts to isolate the inhibitor were unsuccessful due to limited quantities of serum. Strongly inhibitory serum from three mothers of infants with transient familial neonatal hyperbilirubinemia had normal progestin activity as determined by bioassay; however, the method lacks specificity (23). Studies of urinary pregnanediol excretion by these mothers during pregnancy have not been performed.

The inhibitor is probably of maternal origin, as maternal serum at term always produced greater inhibition than was observed with cord blood or neonatal serum, although reduced binding of the inhibitor by fetal plasma has not been excluded. The mothers of infants with transient familial neonatal hyperbilirubinemia were not icteric during pregnancy despite high titers of serum inhibitory activity. Their freedom from jaundice is probably due to their large hepatic functional reserve for transferring bilirubin from blood to bile. Although quantitation of this reserve is lacking in man, a factor of 100 has been suggested (11), and in the rat a factor of at least 40 has been demonstrated (24, 25).

It is postulated that the serum inhibitor factor in these mothers crosses the placenta and inhibits hepatic glucuronyl transferase in the neonate. This inhibition is superimposed on a normally occurring delayed development of the hepatic glucuronide conjugating system, particularly glucuronyl transferase activity. The placenta is believed to transfer unconjugated bilirubin from fetus to mother, whose liver conjugates and excretes bilirubin (26, 27). In transient familial neonatal hyperbilirubinemia, jaundice is observed within hours after birth, increases rapidly, and subsequently subsides within approximately 7 to 15 days if the infant survives. The inhibitor is postulated to decrease hepatic glucuronide formation in the neonate, and serum inhibitory activity is either normal or declines significantly by the fourteenth day of life. By contrast, infants with prolonged unconjugated hyperbilirubinemia associated with breast feeding do not become severely icteric until approximately the fifth to tenth day of life. The inhibitory steroid that has been isolated from mothers' milk is not present in colostrum. This finding may be responsible for the delayed onset of severe neonatal jaundice in this syndrome.

The etiology of the increased serum inhibitory activity in maternal serum is unknown. An acquired abnormality seems unlikely, as drug ingestion is denied and every infant has been affected. There is no evidence for an inherited abnormality, since the mothers were unrelated and there was no family history of severe or prolonged neonatal jaundice.

Further studies are needed to establish the identity of the inhibitor, its source, and mechanism of action. Clinical recognition of the syndrome of transient familial neonatal hyperbilirubinemia should reduce the number of cases of severe neonatal unconjugated hyperbilirubinemia that are currently of unknown etiology. Furthermore, early exchange transfusion should prevent kernicterus in these infants.

Summary

A syndrome of transient familial neonatal unconjugated hyperbilirubinemia not due to known causes has been described in 24 infants of eight unrelated, healthy Caucasian mothers. Four of the infants developed kernicterus.

Beginning in the second trimester of pregnancy, sera from the eight mothers and their newborn infants inhibited direct-reacting bilirubin and *o*-aminophenol glucuronide formation by rat liver slices and homogenates, respectively, four to ten

times more than was observed with sera from a control group of pregnant women and their infants.

The serum inhibitory factor in these women is unidentified. The fact that the inhibitor occurs in pregnancy serum suggests that it is probably a progestational steroid that inhibits glucuronyl transferase activity in the liver of neonates. The mechanism responsible for increased serum inhibitor factor activity in these women is unknown and requires further study.

Acknowledgments

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Appendix

Family G.O. Infant G.O. No. 1 was a full-term female infant whose birth history was benign. On the fifth day of life the infant became deeply jaundiced, and the serum bilirubin concentration was 48 mg per 100 ml. Because the infant was 5 days old and her clinical condition was good, an exchange transfusion was not performed. She is now 7 years old and is normal.

Infant G.O. No. 2, a full-term female infant, had a serum bilirubin concentration of 25 mg per 100 ml on the fourth day of life. The serum bilirubin concentration was 36 mg per 100 ml on the following day, and an exchange transfusion was performed. The child is now 5 years old and is normal.

Family V.H. Infant V.H. No. 2, a 2,977-g infant, was born of an uneventful pregnancy. He was lethargic at birth, and jaundice was noted on the fourth day of life at which time the serum bilirubin concentration was 28 mg per 100 ml and within 4 hours increased to 42 mg per 100 ml. Three exchange transfusions were performed during the subsequent 24 hours. During the last exchange transfusion, he developed a cardiac arrhythmia. Within 12 hours the serum bilirubin again rose to 41 mg per 100 ml. The infant became opisthotonic and had an abnormal Moro reflex. His clinical condition was judged to preclude a fourth exchange transfusion. The serum bilirubin reached a peak of 61 mg per 100 ml on the seventh day of life. He remained opisthotonic and decerebrate throughout his life and had frequent episodes of unexplained fever. He died at 11 months of age during such an episode. Post-mortem examination revealed extensive cerebral atrophy and a histologically normal liver.

A pediatrician who had taken care of infant V.H. No. 1 stated that V.H. No. 1 had been "deeply jaundiced" during the immediate neonatal period. This child is now 8 years old and is healthy.

Infants V.H. No. 3 and 4 were closely observed. Both infants developed high serum bilirubin concentrations. V.H. No. 3 received two exchange transfusions. Both children are now normal.

Family A.C. Infant A.C. No. 1 was considered normal until 80 hours of age at which time the serum bilirubin concentration was 18 mg per 100 ml. An exchange transfusion was performed. The serum bilirubin concentration decreased to 7.5 mg per 100 ml and slowly rose over the subsequent 24 hours to 20 mg per 100 ml, and a second exchange transfusion was performed. Just before the second exchange transfusion, the infant showed the following signs of kernicterus: a high-pitched cry, poor sucking reflex, and opisthotonus. After the second exchange transfusion, the serum bilirubin concentration rose to 18 mg per 100 ml on the fifth day of life and subsided to 12 mg per 100 ml within 24 hours. A.C. No. 1 was a hypotonic infant, but no other abnormalities were noted in the nursery. His development was not normal, and a diagnosis of spastic quadriplegia was made. He is now 6 years old, mentally alert but severely handicapped.

Several months after the birth of A.C. No. 1 we discovered that Mrs. A.C. had received Kynex, a long-acting sulfonamide, for 7 days before delivery. This medication is known to cross the placenta and give persistent blood levels in the newborn infant. It is our hypothesis that the drug interfered with the bilirubin binding capacity of his serum albumin and accounted for the development of a clinical picture compatible with, but not diagnostic of, kernicterus in the newborn period. It is possible that the brain damage he suffered was not due to the hyperbilirubinemia observed in the newborn period; however, the benign birth history and uncomplicated first 3 days of life argue against this explanation.

Infants A.C. No. 2 and A.C. No. 3 demonstrated neonatal jaundice but did not require exchange transfusion, and their development has been normal.

Family D.F. Mrs. D.F. is a sister-in-law of Mrs. A.C. Infants D.F. No. 1 and D.F. No. 2 were premature twins. Their birth histories and first 5 days of life were benign. On the fifth day they became lethargic and jaundiced. D.F. No. 1 had a serum bilirubin concentration of 65 mg per 100 ml. The child's cry was abnormal, and an exchange transfusion was performed. Approximately 12 hours later the infant died . Post-mortem examination revealed kernicterus and normal liver histology. D.F. No. 2 had a serum bilirubin concentration of 40 mg per 100 ml. One exchange transfusion was performed, which reduced the serum bilirubin concentration to 8 mg per 100 ml. On the sixth day of life the serum bilirubin concentration rose to 25 mg per 100 ml and subsequently fell to 14 mg per 100 ml on the eighth day of life. This child is now 7 years of age and is normal.

Mrs. D.F.'s subsequent children have been observed closely for neonatal jaundice. Infant D.F. No. 3 received two exchange transfusions because the serum bili-

rubin concentration rose to 22 mg per 100 ml. D.F. No. 4 and D.F. No. 5 became jaundiced but did not require exchange transfusions. These children are living and normal.

Family H.S. Baby H.S. No. 1, a full-term female infant, was born prematurely after premature rupture of the membranes. The delivery was spontaneous. The infant breathed poorly at birth but improved. She received penicillin and streptomycin when suspected of having sepsis. The infant's course during the first 4 days of life was uneventful, but on the fourth day of life jaundice was noted and serum bilirubin concentration was 31 mg per 100 ml. She became opisthotonic, had irregular respirations, and a reversed Moro reflex. The child died on the sixth day of life. Autopsy revealed kernicterus, intracranial bleeding, and a pulmonary hemorrhage.

Infant H.S. No. 2 manifested hyperbilirubinemia but did not require an exchange transfusion. No follow-up information on this child is available.

Family W.G. Baby W.G. No. 1, a prematurely born male infant, died 36 hours after birth of respiratory distress syndrome. Autopsy revealed massive atalectasis.

Infant W.G. No. 2, a full-term female infant, manifested intense jaundice on the second day of life. The maximal serum bilirubin concentration was 14 mg per 100 ml, which was observed on the fourth day of life. Hyperbilirubinemia slowly subsided during the subsequent 2 weeks.

Infant W.G. No. 3, a full-term male baby, developed icterus at 40 hours of life. The serum bilirubin concentration was 24 mg per 100 ml on the fifth day, and an exchange transfusion was performed. Hyperbilirubinemia disappeared by the twelfth day.

Infant W.G. No. 4 weighed 3,960 g at birth and thrived. The serum bilirubin concentration was 19 mg per 100 ml on the fourth day of life and spontaneously subsided. The child appeared clinically well throughout this period.

Family N.R. Baby N.R. No. 1, a full-term male infant, was well until the second day of life, when intense jaundice was observed. The serum bilirubin concentration rose to a maximum of 18 mg per 100 ml on the fourth day. The infant's clinical condition was good, and exchange transfusion was not performed. Hyperbilirubinemia spontaneously disappeared by the second week of life. This child is now 4 years old and is normal.

Mrs. N.R. became pregnant again in July 1960. Because of the history of unexplained jaundice in her first child, serum specimens were obtained during pregnancy, at term, and postpartum for study of inhibitor factor activity. N.R. No. 2, a full-term male infant, was born after an uneventful delivery and appeared normal. Jaundice became apparent on the second day at which time the serum bilirubin concentration was 12 mg per 100 ml, increased to 16 mg per 100 ml on the fifth day, and spontaneously subsided to normal by the fifteenth day of life. This child is now 3 years old and is normal.

Family J.F. J.F. No. 1, a full-term female infant, appeared clinically normal until the third day of life, when jaundice was observed. The serum bilirubin concentration was 16 mg per 100 ml. Laboratory studies did not reveal evidence of blood group incompatibility, sepsis, or liver damage. The child remained well, and the serum bilirubin concentration rose to 23 mg per 100 ml on the fifth day. An exchange transfusion was performed, and hyperbilirubinemia slowly abated.

Mrs. J.F. became pregnant again in August 1961. Because of the history of unexplained jaundice in her first child, serum specimens were obtained during pregnancy, at term, and postpartum for study of inhibitor factor activity. J.F. No. 2, a full-term male infant, appeared normal at birth. Jaundice became apparent on the third day, and the serum bilirubin was 15.5 mg per 100 ml and increased to 19 mg per 100 ml on the fourth day at which time an exchange transfusion was performed. Hyperbilirubinemia subsequently subsided by the ninth day. This child is now 3 years old and appears normal.

References

1. Day, R., and L. Johnson. Kernicterus. Progr. Hemat. 1959, **2**, 133.
2. Hsia, D. Y. Y., F. H. Allen, Jr., L. K. Diamond, and S. S. Gellis. Serum bilirubin levels in the newborn infant. J. Pediat. 1953, **42**, 277.
3. Brown, A. K., and W. W. Zuelzer. Studies on the neonatal development of the glucuronide conjugating system. J. clin. Invest. 1958, **37**, 332.
4. Lathe, G. H., and M. Walker. An enzyme defect in human neonatal jaundice and in Gunn's strain of jaundiced rats. Biochem. J. 1957, **67**, 9P.
5. Gartner, L. M., and I. M. Arias. Developmental pattern of glucuronide formation in rat and guinea pig liver. Amer. J. Physiol. 1963, **205**, 663.
6. Lucey, J. F., and C. A. Villee. Human fetal hepatic glucuronyl transferase activity. Proceedings of the Tenth International Congress of Pediatrics. Lisbon, 1962.
7. Lucey, J. F., and J. J. Driscoll. Physiological jaundice re-examined *in* Kernicterus, A. Sass-Kortsak, Ed. Toronto, University of Toronto Press, 1961, p. 29.
8. Arias, I. M. The chemical basis of kernicterus. Advanc. clin. Chem. 1960, **3**, 35.
9. Lucey, J., I. Arias, and R. McKay. Transient familial neonatal hyperbilirubinemia. Amer. J. Dis. Child. 1960, **100**, 787.
10. Arias, I. M., and S. Wolfson. Inhibition of bilirubin conjugation *in vitro* by serum from infants with transient familial hyperbilirubinemia and serum from their mothers. Gastroenterology 1960, **38**, 797.
11. Lathe, G. H., and M. Walker. Inhibition of bilirubin conjugation in rat liver slices by human pregnancy and neonatal serum and steroids. Quart. J. exp. Physiol. 1958, **43**, 257.
12. Malloy, H. T., and K. A. Evelyn. The determination of bilirubin with the photoelectric colorimeter. J. biol. Chem. 1937, **119**, 481.

13. Hsia, D. Y., R. M. Dowben, R. Shaw, and A. Grossman. Inhibition of glucuronosyl transferase by progestational agents from serum of pregnant women. Nature (Lond.) 1960, **187**, 693.
14. Levvy, G. A., and I. D. E. Storey. The measurement of glucuronide synthesis by tissue preparations. Biochem. J. 1949, **44**, 295.
15. Crigler, J. F., Jr., and V. A. Najjar. Congenital familial nonhemolytic jaundice with kernicterus. Pediatrics 1952, **10**, 169.
16. Szabó, L., Z. Kovács, and P. Ébrey. Congenital nonhæmolytic jaundice. Lancet 1962, **1**, 322.
17. Arias, I. M. Chronic unconjugated hyperbilirubinemia without overt signs of hemolysis in adolescents and adults. J. clin. Invest. 1962, **41**, 2233.
18. Newman, A. J., and S. Gross. Hyperbilirubinemia in breast-fed infants. Pediatrics 1963, **32**, 995.
19. Arias, I. M., L. M. Gartner, S. Seifter, and M. Furman. Prolonged neonatal unconjugated hyperbilirubinemia associated with breast feeding and a steroid, pregnane-3(alpha),20(beta)-diol, in maternal milk that inhibits glucuronide formation *in vitro*. J. clin. Invest. 1964, **43**, 2037.
20. Arias, I., and L. Gartner. Production of unconjugated hyperbilirubinemia in full term newborn infants following administration of pregnane-3(α), 20(β)-diol. Nature (Lond.) 1964, **203**, 1292.
21. Hsia, D. Y. Y., S. Riabov, and R. M. Dowben. Inhibition of glucuronosyl transferase by steroid hormones. Arch. Biochem. 1963, **103**, 181.
22. Holton, J. B., and G. H. Lathe. Inhibitors of bilirubin conjugation in new-born infant serum and male urine. Clin. Sci. 1963, **25**, 499.
23. Forbes, T. R. Systemic study of plasma progesterone during pregnancy in women and monkeys. Endocrinology 1951, **49**, 218.
24. Weinbren, K., and B. H. Billing. Hepatic clearance of bilirubin as an index of cellular function in the regenerating rat liver. Brit. J. exp. Path. 1956, **37**, 199.
25. Arias, I. M., L. Johnson, and S. Wolfson. Biliary excretion of injected conjugated and unconjugated bilirubin by normal and Gunn rats. Amer. J. Physiol. 1961, **200**, 1091.
26. Schmid, R., S. Buckingham, G. A. Mendilla, and L Hammaker. Bilirubin metabolism in the fœtus. Nature (Lond.) 1959, **183**, 1823.
27. Grodsky, G. M., A. Contopoulos, R. Fanska, and J. Carbone. Distribution of bilirubin-H^3 in the fetal and maternal rat. Amer. J. Physiol. 1963, **204**, 837.