0031-3998/89/2505-0452$02.00/0
PEDIATRIC RESEARCH

Vol. 25, No. 5, 1989
Printed in U.S.A.

Permeability of the Blood Brain Barrier for ^{125}I-Albumin-Bound Bilirubin in Newborn Piglets

CHUL LEE,[1] WILLIAM OH, BARBARA S. STONESTREET, AND WILLIAM J. CASHORE

Departments of Pediatrics, Women and Infants' Hospital of Rhode Island, Brown University Program in Medicine, Providence, Rhode Island 02905

ABSTRACT. Permeability of the blood brain barrier (BBB) for bilirubin and ^{125}I-albumin was studied in 2-d- and 2-wk-old piglets. ^{125}I-albumin was given by bolus at the beginning of each study. Hyperbilirubinemia was produced by an initial bolus infusion of bilirubin and sustained at a plasma bilirubin:albumin molar ratio of approximately 1.0 by continuous infusion of bilirubin for 3 h. During the study period, arterial pH and blood gas tensions, serum osmolarity, and mean arterial blood pressure were within the physiologic range for age in both groups. Serum albumin and serum total and unbound bilirubin concentrations were higher in the 2-wk-old piglets. Brain bilirubin concentrations and permeability (P·S product) of the BBB for bilirubin were higher in the 2-d-old than in the 2-wk-old piglets, but the values of P·S for albumin were not different between the two groups. In 2-d-old piglets, regional brain bilirubin concentrations and permeability of the BBB were higher in subcortical regions (cerebellum and brainstem) than in the cerebral cortex. Regional brain albumin concentrations and BBB permeability to albumin in 2-d-old piglets were higher only in the cerebellum. In all regions, the bilirubin:albumin molar ratio was higher in the brain tissues than in the blood. In 2-wk-old piglets, the brain concentrations and P·S products for bilirubin were lower and the regional differences were less marked than for 2-d-old animals. We conclude that in 2-d-old piglets the blood brain barrier is more permeable to bilirubin than to albumin, that brainstem and cerebellum are more permeable to bilirubin than cortical regions, and that by 2 wk the permeability of the BBB to bilirubin decreases while permeability to albumin remains unchanged. (*Pediatr Res* 25:452–456, 1989)

Abbreviations

BBB, blood brain barrier
P·S, permeability·surface area

Hyperbilirubinemia is one of the most common problems in the newborn infant. Although the mechanism of bilirubin toxicity is not fully understood, kernicterus may result from the entry of the unbound fraction of unconjugated bilirubin into the brain (1, 2). Because in sick infants kernicterus can occur at very low levels of total serum bilirubin (1–3), it is also possible that immaturity of the blood-brain barrier or injury to it may allow entry of bilirubin into the brain (3–6).

Received July 28, 1988; accepted December 13, 1988.
Address for correspondence and reprints William J. Cashore, M.D., Women and Infants' Hospital, 101 Dudley Street, Providence, RI 02905.
[1] Present address Department of Pediatrics, Yonsei University College of Medicine, 134-Shinchondong Seodaemungu, Seoul, Korea.

In most species of animals, hyperbilirubinemia alone fails to cause kernicterus (4–6), even at very high serum bilirubin concentrations (4–6). Kernicterus occurs more readily in asphyxiated animals (4) or in Gunn rats treated with sulfisoxazole (7). The free bilirubin theory as the sole explanation of kernicterus in low birth wt infants has also been challenged by recent observations that hyperosmolar opening of the BBB for albumin-bound bilirubin might play a role in the pathogenesis of kernicterus with serum bilirubin levels below the range of a saturated bilirubin capacity (6, 8, 9).

Ehrlich introduced the concept of a blood brain barrier when he reported in 1885 that aniline dyes injected into the blood stained all tissues except the central nervous system. During development of the BBB, selective mechanisms mature for the uptake or exclusion of various types of molecules in the central nervous system (10, 11). Recently we have observed postnatal maturation of the BBB for unbound bilirubin in newborn piglets (12). Amtorp (13) observed decreased penetration of ^{125}I-albumin into the brain in 30-d-old rats compared to newborn rats. After osmotic opening of the BBB, serum albumin readily enters the brain with regional differences in concentration of albumin-bound dyes (14) or bilirubin (15).

Our hypothesis is that the BBB is more penetrable to albumin-bound bilirubin at birth than later, and that penetration of albumin-bound bilirubin differs with various regions in the brain.

MATERIALS AND METHODS

Twelve piglets were studied: group 1 consisted of six 2-d-old newborn piglets (1.02 ± 0.14 kg, mean ± SD) and group 2 of six 2-wk-old piglets (2.88 ± 0.56 kg, mean ± SD). The piglets remained with the sow until the morning of the study. Surgical procedures were performed under nitrous oxide inhalation (70% nitrous oxide and 30% oxygen) analgesia and local 1% xylocaine anesthesia. Valium (1–2 mg/kg) was also used for mild sedation in both groups. Polyvinyl catheters (internal diameter 0.58 mm, outer diameter 0.9 mm; Bolab Inc., Lake Havasu, AZ) were placed in the femoral vein for infusion of ^{125}I-albumin and bilirubin, the descending aorta for blood pressure monitoring and blood sampling, and the common carotid artery for brain perfusion at the end of the study.

After a 2-h period for recovery from surgery, baseline determinations were made on the awake, unrestrained piglets. Each was then given 40 to 80 μCi of ^{125}I-albumin (New England Nuclear, Boston, MA) infused as a bolus to 2-d- and 2-wk-old piglets, respectively, according to the method described by Ohno *et al.* (16) for substances of low cerebral vascular permeability. After this, an initial loading dose and steady 3-h infusion of buffered bilirubin solution was infused in both groups. In group 1, the rates of initial and steady infusion were 15 mg/kg/10 min and 12 mg/kg/h, respectively. In group 2, these were 21 mg/kg/10 min and 18 mg/kg/h, respectively. The loading and maintenance doses were selected empirically on the basis of previous

studies (12, 15), to achieve and sustain similar circulating bilirubin:albumin molar ratios of approximately 1.0 in both groups. Bilirubin infusion of the same dose by body wt regardless of age in previous studies (12, 15) revealed higher serum bilirubin:albumin molar ratios in 2-d-old piglets with lower serum albumin levels than in 2-wk-old piglets. The buffered bilirubin solution consisted of 18.6% 0.1-N NaOH, 44.4% of 5% porcine albumin (Sigma Chemical Corp., St. Louis, MO) in normal saline, and 37% 0.055-M sodium phosphate buffer. The bilirubin concentration of this solution was 3 mg/mL, and its pH was 8.0. This supersaturated solution had a bilirubin:albumin ratio of 7:1, and in previous studies (8, 12, 15) was found to be stable for the duration of a 3-h study.

Serum albumin, ^{125}I-albumin, total and unbound bilirubin, osmolarity, arterial blood pH, and blood gas tensions, and mean arterial blood pressure were measured during the baseline period and at 60, 120, 150, and 180 min after the onset of the bilirubin infusion. At the end of each study (180 min), the animal was killed with an overdose of sodium thiamylal. To remove the blood from the brain blood vessels, each brain was perfused with normal saline via the common carotid artery for 15 min at a pressure of 60 mm Hg. The brain was removed and divided into equal parts by sagittal section. Each part was divided into frontal and occipital lobe, midbrain, cerebellum, and brainstem. One-half of the brain was used for counting ^{125}I-albumin content, and the other half was used for determining bilirubin levels. Alternate sides were used for the albumin and bilirubin determinations in successive experiments, with no systematic differences between brain halves.

Serum bilirubin was measured by Martinek's modification of the method of Malloy and Evelyn (17), unbound bilirubin by the peroxidase method (18), osmolarity by vapor pressure (Wescor, Inc., Logan, UT), and albumin by the bromcresol green method (19). Blood gas analysis and pH determinations were performed on a Corning 175 blood gas analyzer (Corning Medical, Medfield, MA). Colorimetric determinations were peformed on a Gilford Spectrophotometer (Model 240) (Guilford Instrument Laboratories, Inc., Oberlin, OH) with an automatic recorder (model 6051). Radioactivity of regional brain samples and of the reference blood samples were measured in a gamma well counter (Packard Autogamma Scintillation Spectrometer, Packard Instruments, Downers Grove, IL). Albumin concentrations of brain regions were calculated from the sp act of the serum albumin. Regional brain bilirubin levels were measured by chloroform extraction after homogenization (20). Regional bilirubin-to-albumin molar ratios were calculated.

The P·S product of the BBB was calculated for bilirubin and albumin in each brain region, and expressed in $mL \cdot s^{-1}$, using a formula adapted from the method of Ohno *et al.* (16), as shown:

$$P \cdot S\ (mL \cdot s^{-1}) = \frac{\dfrac{\text{BRAIN BR}}{t,s}}{\text{PLASMA BR} - \dfrac{\text{BRAIN BR}}{0.2}}$$

where P = permeability coefficient (Ohno *et al.*, Ref. 16), S = cm^2/g = brain capillary surface area, BRAIN BR = $\mu g/g$, PLASMA BR = $\mu g/mL$.

In this equation, the P·S product was calculated from the residual concentration of brain bilirubin or albumin (in $\mu g/g$) over time, t, (in s), divided by the plasma concentration, with a correction Brain BR/0.2 for the equilibration of plasma contents with the brain extravascular extracellular space, assumed as 20% of brain wt. This correction was based on previous estimates by Levin *et al.* (21) and Ohno *et al.* (16) for several mammalian species and was used to adjust for the change in gradient from plasma to brain occurring over time by passive diffusion or redistribution of plasma contents between the circulation and the brain extracellular space. With prolonged circulation of a slowly diffusible substance, this modification of the permeability equation is incorporated to account for back diffusion from the extracellular space to the blood.

The mean serum bilirubin and albumin levels were calculated from the plateau values obtained at 60–180 min after the onset of the bilirubin infusion. Whole brain permeability was estimated from the wt-corrected averages of the regional samples. The same procedure was followed to estimate the P·S product for albumin. Calculation of brain albumin content for this purpose was based on the albumin concentration and sp act of the circulating albumin, because the end point of interest was the brain albumin content derived from plasma during the 3-h study.

The relative concentrations of free ^{125}I-albumin in the batches and during the study period were determined by counting the ^{125}I- in the supernatant after treating the batches and serum at 180 min with trichloroacetic acid. Less than 5% free ^{125}I was present in each batch and during the study period.

Titrations for estimation of bilirubin binding to albumin of 2-d- and 2-wk-old piglets were carried out by addition of small amounts of crystalline bilirubin (dissolved in 0.1 NaOH) to aliquots of serum, followed by peroxidase oxidation of the unbound bilirubin, according to a method previously described (22).

Changes within each group were compared using the ANOVA for repetitive measures. Where a significant difference was found, the Dunnett's multiple range t test was used to compare the means to the baseline values (23). Regional differences in brain bilirubin and albumin were analyzed using ANOVA and the Newman-Keuls multiple comparison test. Comparisons between the two different age groups were performed with the unpaired Student's t test; where repetitive measures were compared between the groups, the Bonferroni correction was used (24). Unless otherwise stated, a $p < 0.05$ was considered statistically significant. All values were expressed as mean ± SEM.

RESULTS

As shown in Table 1, serum osmolarity did not change in either group during the experimental period. Osmolarity was slightly higher in group 1 than in group 2, but the difference was significant only at 180 min. Arterial blood pH was the same in both groups and did not change during the study period. Arterial P_{CO_2} did not change in group 1. In group 2, the Pa_{CO_2} fell at 120 and 180 min to values significantly lower than for group 1, but still remained within the normal range. Mean arterial blood pressure was stable throughout the study in both groups and was higher in group 2 than in group 1, consistent with the difference in age. Pa_{O_2} was normal in all piglets.

As shown in Table 2, serum albumin, total bilirubin, and free bilirubin concentrations were higher in group 2 than in group 1 during the study period. Serum ^{125}I-albumin activity was slightly lower in group 2 than in group 1, but the differences were not significant.

Table 3 shows the mean serum and brain bilirubin and albumin concentrations, the molar ratios of bilirubin to albumin in the serum and brain, and the P·S products for bilirubin and albumin. Serum bilirubin and albumin concentrations were approximately twice as high in the 2-wk-old as in the 2-d-old piglets, giving similiar serum bilirubin-to-albumin molar ratios of 1.07 ± 0.1 and 1.05 ± 0.08 for 2-d- and 2-wk-old piglets, respectively. Brain bilirubin concentrations were similar in the two groups. Brain albumin was approximately twice as high in 2-wk-old as in 2-d-old piglets, reflecting the difference in serum albumin concentrations and possible differences in residual extravascular albumin after equilibration of the ^{125}I-albumin. The brain bilirubin to albumin ratios were 4- to 12-fold greater than the serum bilirubin to albumin ratios, and were more than twice as high in 2-d-old piglets as in 2-wk-old piglets (11.9 ± 1.8 *versus* 4.9 ± 0.7, $p < 0.05$). The whole brain P·S product for bilirubin was significantly higher in 2-d-old piglets ($17.6 \pm 3.9 \times 10^{-6}$ *versus* $6.0 \pm 0.8 \times 10^{-6}$ $mL \cdot s^{-1}$, $p < 0.05$); the whole brain P·S products for albumin were the same at both ages.

Table 1. *Serum osmolarity, arterial pH, P_{CO_2}, and mean arterial blood pressure (mean ± SEM) in two groups of piglets*

	Baseline	60 min	120 min	180 min
Serum osmolarity (mosmol/liter)				
Group 1*	295 ± 9	303 ± 10	294 ± 10	303 ± 6
Group 2†	276 ± 3	273 ± 4	281 ± 2	277 ± 3‡
Arterial pH				
Group 1	7.43 ± 0.02	7.43 ± 0.02	7.45 ± 0.01	7.43 ± 0.01
Group 2	7.43 ± 0.01	7.42 ± 0.02	7.44 ± 0.02	7.45 ± 0.01
P_{aCO_2} (mm Hg)				
Group 1	47.9 ± 2.3	45.0 ± 1.6	44.4 ± 0.8	45.9 ± 1.6
Group 2	43.4 ± 1.5	41.8 ± 1.8	38.4 ± 1.6‡	36.8 ± 1.4‡
MABP (mm Hg)§				
Group 1	57.5 ± 2.9	55.5 ± 1.9	58.0 ± 2.1	58.0 ± 2.4
Group 2	71.5 ± 4.1	67.0 ± 2.4	75.2 ± 3.4‡	76.5 ± 3.5‡

* Group 1 = 2-d-old piglets ($n = 6$).
† Group 2 = 2-wk-old piglets ($n = 6$).
‡ $p < 0.01$ compared to 2-d-old piglets.
§ Mean arterial blood pressure.

Table 2. *Serum albumin and ^{125}I-albumin, and serum total and unbound bilirubin (mean ± SEM) in two groups of piglets*

	Baseline	60 min	90 min	120 min	150 min	180 min
Serum albumin (g/dL)						
Group 1*	1.06 ± 0.12	1.13 ± 0.11	ND†	1.07 ± 0.10	ND	1.13 ± 0.12
Group 2‡	1.96 ± 0.11§	2.09 ± 0.14§	ND	2.16 ± 0.18§	ND	2.17 ± 0.16§
Serum ^{125}I-albumin ($\times 10^5$ cpm/mL)						
Group 1	0.04 ± 0.01	9.59 ± 1.32	8.75 ± 1.34	8.48 ± 1.61	8.05 ± 1.90	8.32 ± 2.23
Group 2	0.03 ± 0.01	8.07 ± 1.27	7.20 ± 1.19	6.63 ± 1.19	5.85 ± 0.94	5.44 ± 0.87
Serum total bilirubin (mg/dL)						
Group 1	0.7 ± 0.4	7.3 ± 1.0	9.4 ± 0.9	9.4 ± 0.9	11.3 ± 1.1	12.0 ± 0.9
Group 2	0.6 ± 0.4	13.8 ± 1.3§	15.9 ± 1.0§	18.9 ± 1.1§	22.4 ± 1.6§	24.0 ± 1.3§
Serum unbound bilirubin (nM/liter)						
Group 1	Not detectable	34.0 ± 4.3	37.5 ± 3.8	40.1 ± 3.8	41.0 ± 3.9	46.4 ± 2.9
Group 2	Not detectable	64.3 ± 6.5	69.4 ± 7.3§	86.6 ± 6.6§	91.1 ± 9.5§	95.1 ± 7.7§

* Group 1 = 2-d-old piglets ($n = 6$).
† ND = not determined.
‡ Group 2 = 2-wk-old piglets ($n = 6$).
§ $p < 0.01$ compared to 2-d-old piglets.

Table 3. *Serum and brain bilirubin (BR), albumin (ALB), molar ratios, and P·S products (mean ± SEM)*

	Serum			Brain			P·S Products, $mL \cdot s^{-1}$	
Group	BR (mg/dL)	ALB (g/dL)	BR:ALB ratio	BR ($\mu g/g$)	ALB ($\mu g/g$)	BR:ALB ratio	BR ($\times 10^{-6}$)	ALB ($\times 10^{-7}$)
2 d	9.9 ± 0.9*	1.1 ± 0.1*	1.07 ± 0.1	7.1 ± 1.3	95.2 ± 11.0*	11.9 ± 1.8*	17.6 ± 3.9*	12.6 ± 2.3
2 wk	19.0 ± 1.3	2.1 ± 0.2	1.05 ± 0.08	6.5 ± 0.7	183.5 ± 10.7	4.9 ± 0.7	6.0 ± 0.8	12.9 ± 2.4

* $p < 0.05$ *vs.* 2 wk old.

Figure 1 shows the regional distribution of brain bilirubin and albumin in the cerebellum, brainstem, midbrain, frontal, and occipital lobes. In both groups, the cerebellum and brainstem had higher bilirubin concentrations than the occipital and frontal lobes. Brainstem and cerebellum bilirubin concentrations were significantly higher in 2-d-old than in 2-wk-old piglets, despite lower serum bilirubin concentrations as noted earlier. Regional differences in brain albumin were less prominent (Fig. 1, *lower panel*). The frontal and occipital lobe albumin concentrations were about 2-fold higher in 2-wk-old than in 2-d-old piglets ($p < 0.05$), similar in magnitude to the age-related difference in serum albumin concentration.

Figure 2 shows the regional P·S products for bilirubin and albumin in 2-d- and 2-wk-old piglets. The P·S products for bilirubin in the cerebellum, brainstem, and midbrain were significantly higher in 2-d-old piglets than in 2-wk-old piglets. Brainstem and cerebellum in the 2-d-old group had higher P·S values for bilirubin than midbrain, occipital, or frontal lobe. In the 2-wk-old group, P·S for the brainstem was higher than for the midbrain and frontal lobe, and P·S of the cerebellum was higher than for the frontal lobe as well. The P·S for albumin (Fig. 2, *lower panel*) did not differ between age groups. In 2-d-old piglets, P·S of the cerebellum was higher than for all other regions, and at 2 wk the cerebellum P·S was higher than for brainstem and frontal lobe.

DISCUSSION

The injectate and last serum sample during the study period contained less than 5% free ^{125}I. Because of the low free ^{125}I concentration, the rapid uptake of iodide by the thyroid gland, and the capacity of the choroid plexus to transport free iodide rapidly from the brain even in immature animals (25) our observations are consistent with the uptake of ^{125}I-albumin into the brain, rather than uptake of free brain ^{125}I. Unlabeled porcine albumin was used to stabilize the bilirubin infusate, but because

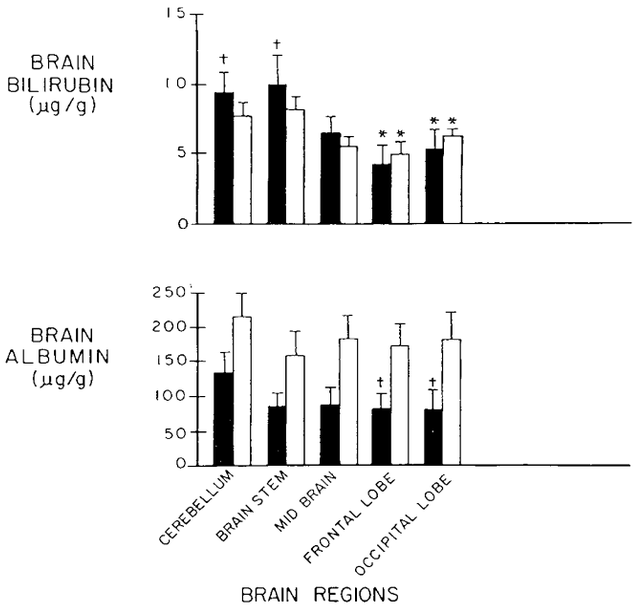

Fig. 1. Regional distribution of bilirubin and ^{125}I-albumin in the brain. *Solid bars*, 2-d-old piglets; *open bars*, 2-wk-old piglets. Mean ± SEM. For within-group and between group comparisons, * $p < 0.05$ compared to cerebellum and brainstem within same age group. † < 0.05 compared to 2-wk-old piglets.

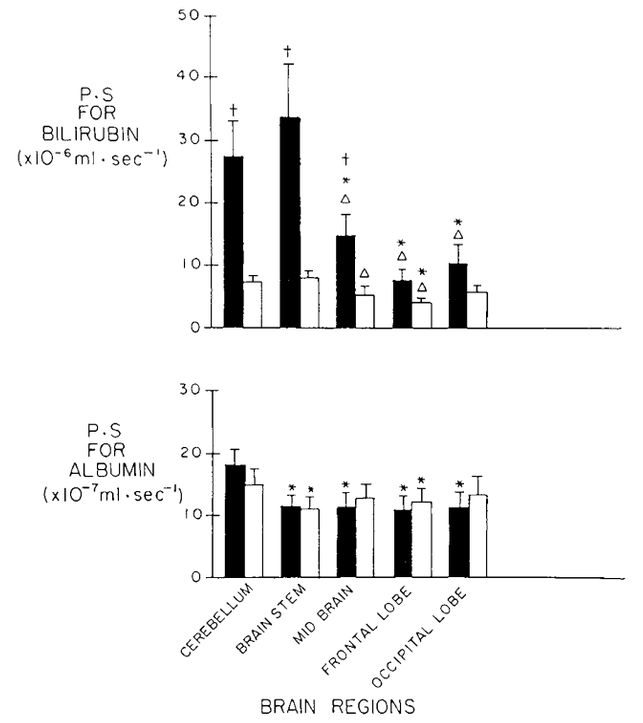

Fig. 2. Regional permeability·surface area (P·S) products for bilirubin and ^{125}I-albumin. *Solid bars*, 2-d-old piglets; *open bars*, 2-wk-old piglets. Mean ± SEM. For within-group and between-group comparison, * $p < 0.05$ compared to cerebellum within same age group. Δ $p < 0.05$ compared to brainstem within same age group. † $p < 0.05$ compared to 2-wk-old piglets.

labeled porcine albumin was not available, human ^{125}I-albumin was used as the tracer for determination of permeability. The structure and mol wt of the two albumins is similar enough that in a short-term experiment and with no previous exposure of the piglets to human protein, this was considered an acceptable procedure.

Recent studies have examined the predictive value for kernicterus of risk factors such as total or free serum bilirubin level, acidosis, and sepsis (1–3, 26). One finding of these studies has been that such risk factors have little prognostic value for kernicterus. Even the total serum bilirubin levels did not differ between low birth wt infants dying with and without kernicterus. Although it has often been postulated that free bilirubin causes kernicterus, there is as yet no conclusive proof for this hypothesis, (9) and Levine *et al.* have suggested the possibility that bilirubin enters the brain while bound to albumin (27).

The present study shows that in young piglets there are no quantitative differences in the BBB to albumin between 2 d and 2 wk of age. The very low P·S product for albumin found in these studies shows that a functional BBB to albumin exists at 2 d of age, with no indication of maturational change during the subsequent 2 wk. This is in agreement with the physiologic and morphologic evidence for a blood brain barrier to albumin in other species, including morphologic observations of the prenatal formation of capillary endothelial tight junctions in the cerebral cortex of the developing rat (28).

The blood brain barrier in the piglet, however, appears more permeable to bilirubin than to albumin, and significantly more permeable to bilirubin at 2 d than at 2 wk. Bilirubin to albumin ratios in the brain were higher than the serum ratios in both age groups, and were significantly higher at 2 d than at 2 wk. Regional differences in brain bilirubin distribution were prominent at 2 d: the finding of higher bilirubin concentrations in the cerebellum and brainstem than in other regions is consistent with our previous studies in young piglets (12, 15), including some animals maintained at higher serum bilirubin to albumin ratios than in the present study (12). At 2 wk, these regional differences were less prominent, although the bilirubin content of the cerebellum was still higher than that of the occipital and frontal lobes. Regional differences in permeability to albumin were less striking, except for the cerebellum. Brain albumin content relative to serum albumin concentration did not change with age, and neither regional nor age-related differences in permeability to albumin can explain the decrease in brain bilirubin uptake associated with increasing postnatal age. These data support the concept of progressive postnatal maturation of the blood brain barrier to bilirubin. Because the P·S products for albumin were the same at both ages, increased barrier permeability to bilirubin in younger animals is unlikely to be a result of increased permeability to albumin in less mature piglets.

The blood brain barrier to albumin may mature earlier than that for bilirubin. Amtorp (13) observed that the concentration of ^{125}I-albumin in brain is higher in newborn than in juvenile rats, and that the brain albumin concentration decreases with increasing age. He suggested that a larger extracellular space in the brain of the newborn rat may contribute to a higher tissue concentration of ^{125}I-albumin. We cannot exclude the possibility that the higher cerebellar concentrations of bilirubin and albumin found in our studies may be due to a larger extracellular space or a richer capillary network in that region. With unilateral osmotic opening of the blood brain barrier in mature animals, Chiueh *et al.* (14) found that the lowest concentrations of ^{125}I-albumin and albumin-bound Evans blue were in the cerebellum and brainstem. The opposite observation in this study suggests that the mechanisms of penetration of albumin and bilirubin into the brain may be different under conditions of intact barrier function or osmotic opening, and that the apparent higher permeability of cerebellum to albumin in our studies is not a result of opening the blood brain barrier. It is, of course, possible that a less mature piglet or a preterm infant might have a less mature blood brain barrier and a higher likelihood of albumin entry into the brain, with a consequent risk of low bilirubin kernicterus due to entry of bound bilirubin—a possibility not addressed by the present study.

Several possible mechanisms could increase the residual brain bilirubin content in less mature animals, even if there were no important age-related differences in the anatomic and transport properties of the barrier for bilirubin. For example, age-related differences in brain composition, *e.g.* in extracellular water and protein, phospholipid content, or state of myelination could account for the retention of bilirubin in marked excess of the measured albumin content in the younger piglets. More rapid clearance of bilirubin from a more mature brain could also explain the lower brain bilirubin content of 2-wk-old piglets. Postmortem degradation of bilirubin within the brains in group 2 is unlikely, because all brains were perfused and placed on ice immediately after each experiment. None of these possibilities negates the observation that at both ages studied and in all regions, residual bilirubin in the brain exceeded the concentration of its albumin carrier even though the barrier to albumin remained constant regardless of age and was presumably intact. Furthermore, the retention of more bilirubin in the brain, at higher concentrations in the subcortical regions, would be consistent with a higher risk of kernicterus at a younger age.

Acknowledgments. The authors acknowledge the excellent technical assistance of Ms. Donna L. A. Piva and Ms. Nancy L. Gelardi. We would also like to thank Ms. Lea M. Gold for preparation of this manuscript.

REFERENCES

1. Cashore WJ, Oh W 1982 Unbound bilirubin and kernicterus in low-birth-weight infants. Pediatrics 69:481–485
2. Stern L, Denton RL 1965 Kernicterus in small premature infants. Pediatrics 35:483–485
3. Kim MH, Yoon JJ, Sher J, Brown AK 1980 Lack of predictive indices in kernicterus: a comparison of clinical and pathologic factors in infants with or without kernicterus. Pediatrics 66:852–858
4. Lucey JF, Hibbard E, Behrman RE, Esquivel FO, Gallardo D, Windle WF 1964 Kernicterus in asphyxiated newborn Rhesus Monkeys. Exp Neurol 9:43–58
5. Chen H-C, Lien I-N, Lu T-C 1965 Kernicterus in newborn rabbits. Am J Pathol 46:331
6. Levine RL, Fredericks WR, Rapoport SI 1982 Entry of bilirubin into the brain due to opening of the blood-brain barrier. Pediatrics 69:255–259
7. Johnson L. Sarmiento F, Blanc WA, Day R 1957 Kernicterus in rats with an inherited deficiency of glucuronyl transferase. Am J Dis Child 97:591–608
8. Bratlid D, Cashore WJ, Oh W 1983 Effect of serum hyperosomolarity on opening of blood-brain barrier for bilirubin in rat brain. Pediatrics 71:909–912
9. Levine RL 1979 Bilirubin: worked out years ago? Pediatrics 64:380–385
10. Purdy JL, Bondy SC 1976 Blood-brain barrier: selective changes during maturation. Neuroscience 1:125–129
11. Pardridge WM, Mietus LJ 1982 Kinetics of neutral amino acid transport through the blood-brain barrier of the newborn rabbit. J Neurochem 38:955–962
12. Lee C, Stonestreet BS, Outerbridge E, Oh W, Cashore WJ 1986 Postnatal maturation of the blood brain barrier for unbound bilirubin in piglets. Pediatr Res 20(suppl):353(abstr)
13. Amtorp O 1976 Transfer of ^{125}I-albumin from blood into brain and cerebrospinal fluid in newborn and juvenile rats. Acta Physiol Scand 96:399–406
14. Chiueh EE, Sun CL, Kopin IJ, Fredericks WR, Rapoport SI 1978 Entry of (^{3}H) norepinephrine, (^{125}I) albumin and Evans blue from blood into brain following unilateral osmotic opening of the blood-brain barrier. Brain Res 145:291–301
15. Burgess GH, Stonestreet BS, Cashore WJ, Oh W 1985 Brain bilirubin deposition and brain blood flow during acute urea-induced hyperosmolarity in newborn piglets. Pediatr Res 19:537–542
16. Ohno KK, Pettigrew D, Rapoport SI 1978 Lower limits of cerebral vascular permeability to non-electrolytes in the conscious rat. Am J Physiol 235:H299–H307
17. Martinek RG 1966 Improved micromethod for determination of serum bilirubin. Clin Chim Acta 13:161–170
18. Jacobson J, Wennberg RP 1974 Determination of unbound bilirubin in serum of newborns. Clin Chem 20:783–789
19. Doumas BT, Watson WA, Biggs HG 1971 Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31:87–96
20. Bratlid D, Winsnes A 1971 Determination of conjugated and unconjugated bilirubin methods based on direct spectrophotometry and chloroform extraction. Scan J Clin Lab Invest 28:41–48
21. Levin VA, Fenstermacher JD, Patlak CS 1970 Sucrose and inulin space measurements of cerebral cortex in four mammalian species. Am J Physiol 219:1528–1533
22. Cashore WJ, Monin PJP, Oh W 1978 Serum bilirubin binding capacity and free bilirubin concentrations: a comparison between Sephadex G-25 filtration and peroxidase oxidation technique. Pediatr Res 12:195–198
23. Winer BJ 1971 Single factor experiments having repeated measures on the same elements. In: Maytham W, Shapiro A, Stern J (ed) Statistical Principles in Experimental Design. McGraw-Hill Book Company, New York, pp 261–308
24. Wallenstein S, Zucker CL, Fleiss JL 1980 Some statistical methods useful in circulation research. Circ Res 47:1–9
25. Robinson RJ, Cutler RWPO, Lorenzo AV, Barlow CF 1968 Development of transport mechanism for sulfate and iodide in immature choroid plexus. J Neurochem 15:455–458
26. Turkel SB, Guttenberg ME, Moynes DR, Hodgman JE 1980 Lack of identifiable risk factors for kernicterus. Pediatrics 66:502–506
27. Levine RL 1983 Bilirubin and blood-brain barrier. In: Levine RL, Maisels MJ (ed) Hyperbilirubinemia in the Newborn. Report of the Eight-Fifth Ross Conference on Pediatric Research. Ross Laboratories, Columbus, pp 125–135
28. Carley WD, Maxwell DS 1970 Development of the blood vessels and extracellular spaces during postnatal maturation of rat cerebral cortex. J Comp Neurol 138:31–48