Morphological changes in the cochlear nucleus and nucleus of the trapezoid body in Gunn rat pups

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Mechanisms underlying bilirubin encephalopathy and hearing loss remain poorly understood, including the way bilirubin enters the nervous system and how bilirubin accumulates in circumscribed regions of the brain. The present experiments examined the auditory brainstem in heterozygous (Nj) and homozygous (jj) Gunn rats at an age when serum bilirubin levels were highest, and after brain bilirubin concentration was artificially raised by sulfadimethoxine administration. In four litters of 11–12 day old Gunn rats, Nj and jj littermates received a single intraperitoneal injection of sulfadimethoxine (100 mg/kg) or a comparable volume of saline. At 16–17 days of age, brainstem auditory evoked potentials were recorded to assess the severity of bilirubin toxicity in the Nj and jj animals. Following the recordings, each animal was perfusion-fixed and frozen sections of the brainstem were cut in the transverse plane from medullary through mesencephalic levels. Sections were mounted on slides, stained with thionin and coded to avoid observer bias. Quantitative analysis revealed no differences between saline and sulfa-treated Nj rats for cochlear nucleus volume, or for cell size in the cochlear nucleus or superior olive. In the sulfa-treated jj rats, cochlear nucleus volume, and cross-sectional areas of spherical cells in the anteroventral cochlear nucleus and principal cells in the nucleus of the trapezoid body, were all significantly smaller than in the combined groups of Nj animals. The affected areas in the cochlear nucleus and superior olive are innervated by large axosomatic end-bulbs of Held or calyceal endings, and were associated with bilirubin staining of glia in the most severely jaundiced jj sulfa-treated rats. The findings suggest that cells receiving synaptic input from end-bulbs or calyces are early targets of bilirubin toxicity in the auditory system.

Bilirubin encephalopathy; Brainstem auditory evoked potentials; Hearing loss; Hyperbilirubinemia; Jaundice; Kernicterus; Trapezoid body; Cochlear nucleus; Superior olive

Introduction

Bilirubin toxicity in premature and low birth weight infants causes multiple neurological deficits ranging from deafness and athetoid cerebral palsy to mild mental retardation and cognitive disturbances (Volpe, 1987). Bilirubin is a breakdown product of hemoglobin and is normally conjugated in the liver to water-soluble glucuronide by the enzyme glucuronyl transferase. Conjugated bilirubin is excreted in bile into the small intestine. Unconjugated bilirubin, which binds to albumin in the blood, can enter the nervous system when the binding capacity of albumin is exceeded (Odell, 1959). In full-term newborns, the activity of hepatic glucuronyl transferase is not fully developed, which, in part, produces a state of 'physiological' jaundice. In premature and low birth weight infants, the activity of this enzyme is still lower and the levels of unconjugated bilirubin may exceed the binding capacity of albumin, resulting in bilirubin encephalopathy (Volpe, 1987).

Bilirubin toxicity affects specific regions of the brain including the cerebellum, basal ganglia, brainstem auditory and vestibular nuclei, and oculomotor nuclei (Jew and Williams, 1977; Johnson et al., 1959; Schutta and Johnson, 1967, 1969, 1971; Schutta et al., 1970). Why bilirubin only affects some regions of the nervous system and not others remains unknown. In experimental studies of the effects of bilirubin on the auditory system, the cochlear nucleus is stained with yellow bilirubin pigment and shows degenerative changes associated with hyperbilirubinemia (Jew and Williams, 1977; Jew and Sandquist, 1979; Rose and Wisniewski, 1979). Postmortem examinations of jaundiced infants revealed bilirubin deposits in the cochlear nucleus, superior olive, nuclei of the lateral lemniscus and inferior colliculus (Ahdab-Barmada and Moossy, 1984; Dublin, 1951, 1986). In clinical investigations, however, factors contributing directly to bilirubin encephalopathy have been difficult to study in isolation from other...
toxic influences typically found in neonatal intensive care units (e.g., hypoxia, acidosis, aminoglycoside exposure; see Bergman et al., 1985; Salamy et al., 1989).

Most anatomical investigations have been limited to the analysis of individual auditory structures in jaundiced animals with no attempt to survey across regions to determine which areas are affected, and which appear unaffected at a given age (e.g., Jew and Williams, 1977; Jew and Sandquist, 1979). Evoked potential studies have implicated the involvement of multiple brainstem auditory nuclei in young jaundiced rats (e.g., Shapiro, 1988; Shapiro and Hecox, 1988; Shapiro and Hecox, 1989), but these findings were not correlated with anatomical measures of brainstem pathology. A more comprehensive understanding of bilirubin toxicity in the auditory system may be provided by systematic experimental studies on the effects of hyperbilirubinemia across several brainstem auditory nuclei. Determining if multiple auditory nuclei are affected at a given age, and whether some regions appear affected before others, would suggest possible ways in which bilirubin produces hearing loss. Such investigations could help to differentiate between the degenerative effects largely due to bilirubin exposure and those due to secondary transneuronal events.

The present experiments examined the structure and function of the auditory brainstem in heterozygous and homozygous Gunn rats at an age when serum bilirubin levels are normally at their peak (Johnson et al., 1959; Schutta and Johnson, 1969), and after the concentration of bilirubin in the brain was artificially raised by sulfadimethoxine administration (Diamond and Schmidt, 1966). This study is the first in a series to quantitatively compare structures in the central auditory pathway of jaundiced and heterozygous Gunn rats, and to associate anatomical changes with evoked potential measures of brainstem pathology in the same animals.

Materials and Methods

Experiments were conducted on heterozygous (Nj) and homozygous recessive (jj) Gunn rats from four separate litters. Gunn rats that are homozygous recessive for the jaundiced condition lack the enzyme glucuronyl transferase and are noticeably jaundiced from abnormally high levels of unconjugated bilirubin in their blood. Heterozygotes have 50% of normal activity for this enzyme, but their serum bilirubin levels are normal (Strebel and Odell, 1971).

Each litter consisted of 5–8 jj and 5–6 Nj animals. Homozygous Gunn rats were distinguished from heterozygous littermates during the early postnatal period by their yellow color and were identified with an ear punch. To help control for the effects of interlitter variability, Nj-jj pairs were matched for weight and randomly assigned to receive saline or sulfadimethoxine.

At 11 or 12 days of age, a single intraperitoneal injection of 100 mg/kg sulfadimethoxine (Albon, Hoffmann-La Roche Inc.; 400 mg/ml diluted twenty-fold with normal saline) or a comparable volume of saline without sulfonamide was administered. Sulfadimethoxine competes with bilirubin binding sites on albumin and promotes the net transfer of bilirubin into brain (Diamond and Schmidt, 1966). At 16–17 days of age, each animal was reweighed, anesthetized with ketamine (60 mg/kg ip) and acepromazine (6 mg/kg ip), and brainstem auditory evoked potentials (BAEPs) were recorded to assess the severity of bilirubin toxicity. Procedures used to record BAEPs were similar to those described previously (Shapiro and Hecox, 1989) and the results are published in a separate report (Shapiro and Conlee, 1991). Upon completion of BAEP recordings, each animal was given an overdose of sodium pentobarbital (Nembutal) and perfused transcendially with physiological saline followed by a solution containing 1.0% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). Each brain was dissected from the skull and placed in fresh fixative for 2–3 days. Each brainstem was then blocked transversely in a plane perpendicular to the floor of the fourth ventricle and immersed in fixative containing 30% sucrose until it sank. After sucrose infiltration, frozen sections were cut serially at 30 microns in the transverse plane from medullary through mesencephalic levels. Semi-adjacent sections were mounted sequentially on slides and stained with thionin. Slides were examined qualitatively to survey the distribution of yellow staining in the brainstems of control and sulfato-treated Nj and jj animals. To avoid bias, slides were coded before morphometric comparisons were made and examined without observer knowledge of the genotype or treatment group.

Brainstem auditory structures chosen for quantitative analysis included the overall volume of the cochlear nucleus, two cell types in the ventral cochlear nucleus, and third-order neurons in both the medial superior olive (MSO) and medial nucleus of the trapezoid body (MNTB). In the ventral cochlear nucleus, both large spherical cells and globular cells were measured. Large spherical cells were located in the rostral pole of the anteroventral cochlear nucleus (AVCN), while globular cells were found at the junction of the anterior and posterior subdivisions of the ventral cochlear nucleus, distributed among bifurcating cochlear nerve fibers (Harrison and Warr, 1962; Osen, 1969; Brawer et al., 1974; Webster and Trun, 1982). To control for possible variation in cell size along the longitudinal axis of each nucleus, sections containing both the MSO and MNTB were chosen for quantitative study at levels...
corresponding to the 25th and 75th posterior-to-anterior percentiles through the MSO. In two semi-adjacent sections through the cochlear nucleus or superior olivary complex, the outline of each neuron with a stained nucleolus was drawn at ×1630 with a camera lucida. Drawings were measured planimetrically using a Bioquant 4 image analysis system. To determine cochlear nucleus volume, the outline of the cochlear nucleus in alternate sections was traced for each animal at ×113 with a camera lucida. These values were measured using the image analyzer, summed, divided by the square of the linear magnification, and multiplied by both the inverse of the sampling interval (i.e., 2) and section thickness (30 microns) to yield total nuclear volume.

In some animals, the ratios of glia to neurons and the percentages of yellow bilirubin-stained cells were estimated in both the MNTB and in the large spherical cell area of the AVCN. For each region, neurons and glia were counted in five randomly selected locations in each of two semi-adjacent sections using an ocular grid reticule with an area of 0.1 mm² at ×1000 magnification (Ling and Leblond, 1973). Endothelial cells were excluded from the counts, and glia were not differentiated according to subclass. The total number of glial cells in ten samples taken from two sections was divided by the total number of neurons to give the ratio of glial cells-to-neurons in each nucleus. In each region, the number of yellow stained glia was divided by the total number of glia in that region to yield the percentage of ‘kernicteric’ glial cells.

Initially, the means for saline- and sulfa-treated Nj rats were compared. Because there were no significant differences in cochlear nucleus volume or in the mean cross-sectional areas of the brainstem auditory neurons in these controls, those data were combined for subsequent comparisons. A multifactorial analysis of variance (SPSS MANOVA software) was performed to compare the combined Nj data with the jj sulfa-treated group across the five dependent variables (cochlear nucleus volume, and mean cross-sectional area of globular cells and spherical cells in the AVCN, and MNTB and MSO neurons). Post hoc comparisons were made between groups using a one-factor analysis of variance on each variable; the probability values given are for two-tailed tests. The percent of total body weight gain was compared between the Nj and jj animals using a t-test.

Results

At 11–12 days of age when saline or sulfadimethoxine was first administered, overall body weight of 18.4 ± 2.4 (mean ± S.D.) did not differ significantly between Nj and jj animals (Table I). When animals were sacrificed at 16–17 days, mean percentage gain in body weight per day was 10% for the Nj saline group, 8.9% for the Nj sulfa rats, 8.3% for the saline controls and 3.9% for jj animals given sulfonamide (Table I). The percentage of daily weight gain for the sulfa-treated jj animals was significantly less than for the saline-treated jj animals (t₁₄ = 2.98, P < 0.01), or for either of the Nj groups (Table I).

Group means were used to construct histograms for cochlear nucleus volume (Fig. 1), and neuron cross-sectional area (‘cell size’) in the cochlear nucleus and superior olive (Fig. 2). For the Nj rats, there were no statistically significant differences between saline and sulfa-treated groups for cochlear nucleus volume, or in the mean cell size of any of the brainstem auditory neurons. Therefore, values from the two groups of Nj rats were combined for subsequent comparison with the jj animals. Since there was a difference between jj saline and jj sulfa-treated groups in large spherical cell area (F₅,₁₄ = 8.33, P < 0.02), the data from these groups were not combined.

Results of the multivariate analysis for all anatomical variables demonstrated an overall group difference between all Nj rats and the jj animals given sulfadimethoxine (F₅,₁₄ = 5.15, P < 0.007). Post hoc comparisons revealed that several auditory structures were
significantly smaller in the jj sulfa-treated Gunn rats than those in the combined Nj sulfa and saline groups (Figs. 1 and 2). Mean volume of the cochlear nucleus in the sulfa-treated jj animals \((949 \pm 23 \times 10^6 \mu m^3)\) was reduced by about 8% compared to cochlear nucleus volume in the Nj rats \((1,034 \pm 17 \times 10^6 \mu m^3)\) \((F_{18} = 8.07; P < 0.01)\). Of the two cell types measured in the ventral cochlear nucleus, large spherical cells in the jj sulfa-treated rats were 12% smaller than those in the Nj animals \((238.7 \pm 7.4 \mu m^2 \text{ vs. } 269.8 \pm 6.2 \mu m^2)\) respectively, \(P < 0.005\); globular cells did not differ significantly between these groups. In the superior olivary complex, principal cells in the MNTB of the jj sulfa-treated animals were also 12% smaller than MNTB neurons in the combined Nj drug and saline groups \((260.1 \pm 5.1 \mu m^2 \text{ vs. } 295.7 \pm 10.0 \mu m^2)\) respectively, \(P < 0.008\); MS0 neurons did not differ statistically between these groups. There were no statistically significant differences in any of the anatomical measures between the jj saline group and the Nj animals (Figs. 1 and 2).

Yellow staining was readily apparent in the AVCN and MNTB of the most severely affected of the jj sulfa-treated animals. In three such animals, yellow bilirubin pigment stained both astrocytes and oligodendrocytes, but was rarely seen in neurons. In areas outside of the cochlear nucleus and MNTB, yellow pigment was seldom observed in neurons or glia of the jj sulfa-treated animals; yellow pigment was absent from the brainstems of the jj saline animals and from both groups of Nj rats. Thus, those regions in the cochlear nucleus and superior olive having significantly smaller neurons were also associated with the greatest degree of yellow stained glia in the most severely affected sulfa-treated jj animals. The yellow bilirubin pigment was confined to the glial cell population of each nucleus and not found in neurons.

Other locations where yellow bilirubin pigment was seen in the jj sulfa-treated rats included the cerebellar granule cell layer, the dorsal cochlear nucleus, the posteroverentral cochlear nucleus, the nucleus of the lateral lemniscus, the inferior colliculus, the lateral and medial vestibular nuclei, the spinal nucleus of the trigeminal nerve, and the oculomotor nucleus. Yellow staining in each of these nuclei was sporadic and was associated with both glia and neurons of each region. Although cerebellar Purkinje cell degeneration was pronounced in all saline and sulfa-treated jj Gunn rats, yellow staining of the cerebellum was much less frequently observed in the saline-treated jj rats, and never to the degree shown in the cochlear nucleus and MNTB of the most severely affected jj sulfa-treated rats.

A quantitative description of the percentage of yellow-stained glia and of the ratio of glia-to-neurons (G:N ratio) was made in the MNTB of five Nj saline and five jj sulfa-treated animals from three litters, and in the large spherical cell region of the AVCN in two jj saline and two jj sulfa-treated animals from one litter. In both the MNTB and AVCN, the mean G:N ratio did not differ between the jj sulfa-treated animals and the other groups being compared. The mean G:N ratio in the MNTB was 3.4 in the jj sulfa-treated animals and 3.6 in the Nj saline controls (Table II). Ratios of G:N in the AVCN averaged 3.8 for the sulfa-treated jj rats and 3.9 for the jj saline animals from the same litter. Although mean G:N ratios were not different between the groups, rats with bilirubin staining in the MNTB displayed smaller G:N ratios (Table II). Thus, two jj sulfa treated rats with 19 and 26% bilirubin-stained glia in the MNTB (i.e., rat 47.4 and rat 47.8) had G:N ratios which averaged 2.2. Other jj sulfa-treated animals without bilirubin-staining in the MNTB had G:N ratios which averaged 4.2 (Table II). The difference between G:N ratios for the
two rats with abundant yellow-stained glia and those without was statistically significant ($t_7 = 4.09$, $P < 0.01$). A similar trend was not evident in the AVCN, where the mean G:N ratio was 3.8 for both jj saline and jj sulfa-treated rats. No yellow stained glia were seen in the AVCN of the jj animals given saline, whereas about 13% of glia were stained with yellow bilirubin pigment in the jj sulfa-injected rats (Table II).

**Discussion**

This is the first quantitative study of auditory structures in the brainstem of jaundiced Gunn rats. The volume of the cochlear nucleus and the mean cross-sectional area of neurons in the AVCN and MNTB were significantly smaller for jj sulfa-treated rats than for jj littermates given saline, or for Nj littermates given sulfa or saline. Cross-sectional areas of other neurons measured in the cochlear nucleus or superior olivary complex of jj sulfa-treated animals were not significantly different from those in the other groups studied. In the most severely jaundiced animals from one litter, affected regions also displayed selective yellow staining of glial cells. Except for these animals, very little bilirubin staining was seen anywhere in the brainstems of the remaining animals, including the jj sulfa-treated rats from the other litters examined. Thus, not all auditory neurons in the brainstem of the jj sulfa-treated rats appeared to be affected, and the clearest example of bilirubin-stained elements in the nervous system was confined to glial cells in the most severely affected jj animals.

Converging lines of evidence indicate that a specific class of cells appears to be affected in the ventral cochlear nucleus of jaundiced Gunn rats. In ultrastructural studies, only large neurons in the AVCN were found to be abnormal and to contain enlarged mitochondria, distended endoplasmic reticula, and accumulations of intracellular glycogen (Jew and Williams, 1977; Jew and Sandquist, 1979). BAEP and in-vitro recordings suggest that there may be two populations of postsynaptic neurons in the AVCN, one with normal and one with prolonged synaptic delays (Shapiro, 1988; Shapiro and Hecox, 1988; Zhang et al., 1989). The present results further indicate that not all neurons are affected, at least initially, in the ventral cochlear nucleus of Gunn rats, and that the spherical cells appear to be influenced before other cell types. Although the globular cells did not appear to be affected in the younger Gunn rats, older jaundiced animals do show significant decreases in globular cell size (Brugge et al., 1987; Coleman, Brugge and Shapiro, unpublished observations). Both the overall reduction in cochlear nucleus volume of the younger sulfa-treated jj rats, and a significantly decreased cell size for globular cells in adults, suggest that other regions of the cochlear nucleus become involved as the central effects of hyperbilirubinemia progress.

The anatomical changes found in the present study were positively correlated with abnormalities in the BAEPs recorded from the same animals (see Shapiro

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>GLIA AND NEURON COUNTS IN THE BRAINSTEM OF SELECTED GUNN RATS</th>
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<tr>
<td>Nucleus/Group</td>
<td>Rat No.</td>
</tr>
<tr>
<td>MNTB</td>
<td></td>
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<tr>
<td>Nj saline</td>
<td>44.6</td>
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<tr>
<td></td>
<td>44.7</td>
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<tr>
<td></td>
<td>47.9</td>
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<td></td>
<td>47.10</td>
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<tr>
<td>jj sulfa</td>
<td>48.8</td>
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<tr>
<td></td>
<td>44.3</td>
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<td></td>
<td>44.5</td>
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<td>47.4</td>
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<td>jj saline</td>
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<td></td>
<td>47.5</td>
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<tr>
<td>jj sulfa</td>
<td>47.4</td>
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<td>47.8</td>
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a Litter and rat identification number, e.g., 44.6 represents litter 44, rat number 6.

b Total numbers of cells were determined by counting glia and neurons and summing across ten separate 0.1 mm² areas in each nucleus. See Materials and Methods section.

c Glia to neuron ratio.
were positively correlated with amplitude reductions in waves II and III (Shapiro and Conlee, 1991). These within the brainstem auditory pathways. In the visual system, reduced cell size in the lateral geniculate nucleus of the trapezoid body were reduced in amplitude and prolonged in latency in the jj sulfa-treated rats when compared to the responses from both Nj groups or the jj saline-treated animals. Previously, correlations have been made between focal abnormalities in the BAEP and discrete anatomical changes in the superior olive of humans with confirmed brainstem pathology (Starr and Hamilton, 1976; Chiappa and Ropper, 1982), and in animals with experimental (Wada and Starr, 1983) and naturally occurring lesions (Conlee et al., 1984). In these studies, degeneration (Wada and Starr, 1983) or significantly reduced cell size (Conlee et al., 1984) in the superior olivary complex was correlated with amplitude attenuation in wave III of the BAEP (Creel et al., 1983). In the present investigation, significant reductions in cochlear nucleus volume, and in cell size in the cochlear nucleus and MNTB, were positively correlated with amplitude reductions in waves II and III (Shapiro and Conlee, 1991). These results suggest that components of the BAEP are sensitive indicators of cell size and morphological integrity within the brainstem auditory pathways. In the visual system, reduced cell size in the lateral geniculate nucleus has been shown to be highly correlated with functional measures of amblyopia and the loss of cortical binocularity (Tremain and Ikeda, 1982). A decline in both the size and functionality of sensory neurons may involve diminished protein synthesis (Trune et al., 1987), and appears to be reflected in reduced cell area and histological characteristics (Born and Rubel, 1985; Coleman and O'Connor, 1979) in Nissl-stained material.

Several factors in addition to hyperbilirubinemia play significant roles in the development of bilirubin encephalopathy. The regional metabolic rate is one component that appears to influence the selective accumulation of bilirubin in different areas of the brain (Schutta and Johnson, 1969). Some of the most metabolically and electrically active regions of the nervous system are those that have been commonly associated with kernicterus (Friede, 1975; Zimmerman and Yannet, 1933). These include multiple brainstem nuclei, the cerebellum, the basal ganglia, and the hippocampus. Other recent evidence indicates that bilirubin neurotoxicity may act through glutaminergic excitotoxicity in the hippocampus (MacDonald et al., 1990a,b). Several of these areas, including the cochlear nuclei and superior olivary complex, receive their predominate excitatory input from afferents which utilize L-aspartate, L-glutamate or related amino acids as neurotransmitters (Wenthold, 1985; Godfrey et al., 1988; Collingridge and Lester, 1989). Putative glutaminergic inputs to the spherical cells in the AVCN and principal cells in the MNTB are provided by the large axosomatic endbulbs of Held and calyceal endings (e.g., Harrison and Warr, 1962; Morest, 1968; Ryugo and Fekete, 1982). Cross-sectional areas for both cell types were significantly reduced in the sulfa-treated jj animals, while other neuron classes in the AVCN and superior olive receiving innervation from much smaller boutonal endings were not similarly affected. These results suggest that cells receiving endbulbs or calyces in the AVCN and MNTB are uniquely sensitive to bilirubin toxicity, possible due to the synaptic relationship imposed by these unusually large afferent endings and the powerful excitatory drive they exert postsynaptically (Ryugo and Fekete, 1982).

In addition to the level of metabolic activity, several other factors have been reported to contribute to bilirubin encephalopathy. The high concentration of gangliosides and sphingomyelin in the synaptic membrane, and small acidic changes in pH (0.2–0.4 pH units), have been shown to positively influence the affinity of nervous tissue for bilirubin (Vazquez et al., 1988). Anoxia during the perinatal period can also result in acidosis, which increases cellular uptake of bilirubin (Odell, 1970), and has been suggested as a critical risk factor underlying bilirubin encephalopathy (Vazquez et al., 1988). Anoxic damage to the blood-brain barrier is also believed by some to be necessary for bilirubin neurotoxicity to occur (Chen et al., 1965; Lucey et al., 1964). In the present study, jj sulfa-treated animals gained significantly less weight than did the other groups during the period following sulfa or saline administration. The most severely jaundiced of the jj sulfa-treated rats gained no weight and were near death at the time of sacrifice. The glia in the AVCN and MNTB of these animals were intensely yellow stained and the reduction in the glia-to-neuron ratio in the MNTB was statistically significant. If these particular animals were acidotic, a condition found in other morbid kernicteric animals (Schutta and Johnson, 1969), then the selective accumulation of bilirubin in the glia of these two regions may have resulted from acidosis during hyperbilirubinemia. Other studies have shown that hyperbilirubinemia alone produces a reduction in glial cell number (Sturrock and Jew, 1978), and that anoxia and acidosis during experimental hyperbilirubinemia result in pervasive bilirubin staining of glia and neurons (Chen et al., 1965; Lucey et al., 1964). Our present findings are consistent with both of these earlier reports, but further suggest that glia may selectively accumulate bilirubin, and that glial uptake of bilirubin is localized to regions in the brain stem associated with changes in neuronal size and function. Reduction of cochlear nucleus volume (t15 = 1.82, P < 0.05, one-tailed), and cell sizes of spherical cells in the AVCN (t15 = 2.45, P < 0.05, two-tailed) and principal neurons in the MNTB (t15 = 2.19, P < 0.05, two-tailed) were still observed even when the most severely jaun-
diced animals (from litter 47) were excluded from the analysis. Thus, the role played by bilirubin-stained glia in bringing about the observed neuronal changes remains uncertain.

The results support several earlier reports of anatomical and physiological abnormalities in the cochlear nucleus and superior olivary complex of jaundiced Gunn rats (eg., Jew and Williams, 1977; Jew and Sandquist, 1979; Shapiro and Hecox, 1988, 1989; Uziel et al., 1983). The anatomical changes observed in the auditory system, although small, were statistically significant, and their distribution corresponded to the location of generators for BAEP components shown to be abnormal in the same animals (see Shapiro and Conlee, 1991). In the cerebellum, Purkinje cell degeneration was pronounced, but little evidence of yellow bilirubin staining was seen in any region of this structure. The extreme vulnerability of Purkinje cells in young Gunn rats is firmly established (Schutta and Johnson, 1969; Sawasaki et al., 1976). Yet, the inconsistent association between bilirubin staining and cellular injury in the cerebellum has drawn into question the precise role bilirubin plays in cerebellar neurotoxicity (Volpe, 1987). In the auditory system, little evidence of neuronal degeneration was found, but prevalent yellow-staining was observed only in the most severely jaundiced animals in those auditory nuclei which manifested quantitative changes in neuronal size. This observed pattern of effects indicates that separate neural regions may be uniquely influenced by bilirubin at different times, and that the auditory effects are initiated later than those in the cerebellum. Whether any further changes take place in the auditory system of jaundiced Gunn rats during maturation remains to be shown. Investigating the sequence of auditory changes that result from hyperbilirubinemia, and relating these changes to developmental influences such as age and maturation within the auditory pathways, may suggest mechanisms by which bilirubin selectively affects particular areas of the brain.

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