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Development of adrenal cortical zonation and expression of key elements of adrenal androgen production in the chimpanzee (*Pan troglodytes*) from birth to adulthood

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ABSTRACT

The basis for the pattern of adrenal androgen production in the chimpanzee, which resembles that of humans, is poorly defined. We characterized the developmental zonation and expression of elements of the androgen biosynthetic pathway in the chimpanzee adrenal. The newborn adrenal contained a broad fetal zone (FZ) expressing CYP17, SULT2A1, and Cytochrome B5 (CB5) but not HSD3B; the outer cortex expressed HSD3B but not SULT2A1 or CB5. During infancy, the FZ involuted and the HSD3B-expressing outer cortex broadened. By 3 years of age, a thin layer of cells that expressed CB5, SULT2A1, and CYP17 adjoined the medulla and likely represented the zona reticularis; the outer cortex consisted of distinct zonae fasciculata and glomerulosa. Thereafter, the zona reticularis broadened as also occurs in the human. The adult chimpanzee adrenal displayed other human-like characteristics: intramedullary clusters of reticularis-like cells and also a cortical cuff of zona fasciculata-like cells adjoining the central vein.

1. Introduction

During human fetal development, a specialized zone of the adrenal cortex, known as the fetal zone, comprises about 80% of the mass of the adrenal and produces the large quantities of DHEA and DHEA-sulfate that serve as the principal precursors for placental estrogen formation. The fetal zone of the human adrenal contains large quantities of the enzyme DHEA-sulfotransferase (SULT2A1) and little if any 3β-hydroxysteroid dehydrogenase (HSD3B2) that could direct the adrenal steroid pathway toward delta-4 steroids such as aldosterone and cortisol rather than DHEA/DHEA-sulfate (Barker et al., 1995; Dupont et al., 1990; Mesiano et al., 1993; Parker et al., 1994, 1995). In fact, there appears to be little HSD3B2 in the fetal adrenal until the latter stages of gestation where it is primarily localized to the outer cortex known as the neocortex (Dupont et al., 1990; Mesiano et al., 1993; Parker et al., 1995). The enzyme 17α-hydroxylase/17,20-lyase (CYP17), which can catalyze both the 17α-hydroxylation of pregnenolone and progesterone as well as conversion of such 17-hydroxylated steroids to DHEA and androstenedione, respectively, is characteristic of the fetal and adult human adrenal (Mesiano et al., 1993; Dharia et al., 2005). Cytochrome B5 (CB5), which acts as an accessory protein to CYP17 and promotes its 17,20-lyase activity (Katagiri et al., 1995; Lee-Robichaud et al., 1995), is co-localized with CYP17 in both the fetal zone of the fetal adrenal and the zona reticularis (Dharia et al., 2005), which develops during adrenarche and persists throughout adulthood, in the human adrenal (Dhom, 1973).

Although adrenal androgen production during fetal development and/or during adult life is not common among other mammals, many primate species have been found to bear similarities in the potential for adrenal androgen synthesis to that of the human. Those species that have been found to possess a fetal zone like that of the human also appear to rely on the fetal adrenal to a variable degree to supply precursors for placental estrogen formation during pregnancy (Albrecht et al.,1980; Lanman, 1961; Walsh et al., 1979a, 1979b). Interestingly, however, there is limited information about the morphologic and functional zonation of the fetal and postnatal adrenal of our genetically closest primate relative, the chimpanzee (*Pan troglodytes*).

The available data are suggestive that in the chimpanzee, the fetal adrenal is composed of a morphologically distinct fetal zone and a neocortex (Czekala et al., 1983) and estrogen production during pregnancy follows a similar time course to that of humans

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(Czekala et al., 1983; Reyes et al., 1975; Smith et al., 1999). However, we are not aware of any studies demonstrating the capacity for androgen production by the fetal adrenal or a role for the fetal adrenal in estrogen formation in chimpanzee pregnancy. There are developmental increases in circulating levels of DHEA and DHEA sulfate during adolescence that resemble that seen in human adrenarche (Bernstein et al., 2012; Collins et al., 1981; Copeland et al., 1985; Smail et al., 1982), and there is maintenance in serum levels of DHEA and DHEA sulfate during young adulthood that are within the general range of circulating concentrations noted for humans (Bernstein et al., 2012). Since it is known that DHEA and DHEA sulfate are produced in castrated chimpanzees and are responsive to ACTH (Albertson et al., 1984), these steroids must arise from the adrenal gland. Despite such findings, there are no data concerning the functional phenotype of the adrenal cortical zones of the fetal or postnatal chimpanzee. Based on these limited findings, we sought to better characterize the steroidogenic potential of the adrenal of the fetal, infant, adolescent, and adult chimpanzee by evaluating the zonation and expression of several key elements of the steroidogenic pathway.

2. Methods

Archival adrenal samples from 27 chimpanzees (13 male, 14 female) ranging in age from term newborn to 31 years of age were kindly provided to us by the Yerkes National Primate Research Center and the Southwest National Primate Research Center. At the time of routine autopsy of the chimpanzees, tissues, including the adrenal glands, were removed and were fixed in 10% neutral buffered formalin. Tissues were processed using standard techniques and embedded in paraffin. For our study, five micron sections were mounted on plus slides (corning) and were either stained with hematoxylin and eosin for general morphologic characterization or were utilized for immunohistochemistry using standard techniques in our laboratory.

The anti-human SULT2A1 antiserum was developed in the rabbit against purified human liver DHEA sulfotransferase by Dr. Charles Falany (Department of Pharmacology, University of Alabama at Birmingham). The anti-human CB5 antiserum was developed in the rabbit by Dr. Alan J. Conley (School of Veterinary Medicine, Univ. California at Davis), utilizing recombinant human CB5 donated by Ron Estabrook and Manju Shet, (Univ Texas Southwestern Medical School, Dallas TX). The anti-human HSD3B antiserum (which we find to react with both isozymes of the human, HSD3B1 and HSD3B2, which share 95% sequence homology and are both coded for on chromosome 1 in the human) was developed in the rabbit by Dr. Richard Parker against purified human placental 3β-hydroxysteroid dehydrogenase/delta 5–4 isomerase kindly donated by Dr. James Thomas (Mercer University School of Medicine, Macon GA). The mouse monoclonal anti-human CYP17 antibody was developed by Dr. Richard Parker against recombinant human CYP17 that was generated by an expression plasmid kindly donated by Michael Waterman (Vanderbilt University, Nashville TN). Chimpanzee CYP17 differs by only two amino acids from that in the human, and there is 95% homology between these and the enzyme in rhesus and baboon (Arlt et al., 2002). The antisera used in this study of the chimpanzee adrenal have been previously utilized in several published studies on human and rhesus steroidogenic tissues (Dharia et al., 2004; Mapes et al., 2002; Parker et al., 1995; Parker et al., 2000b, for example).

Briefly described, after the tissue sections were subjected to deparaffinization in xylene and progressive hydration in ethanol, 95% ethanol, 75% ethanol, and distilled water, the sections were incubated with 3% hydrogen peroxide for 10 min at 25 °C to block endogenous peroxidase. Then, sections were rinsed in distilled water and Tris buffer, pH 7.6, and incubated for 20 min at 25 °C in 5% goat serum to block non-specific antibody binding. The sections were then drained and incubated for 1hr at 25 °C with antisera diluted in PBE buffer, ph 7.6 (0.1 M phosphate buffer that contained 1% bovine serum albumin, 1 mM EDTA, 1.5 mM sodium azide). The dilutions of antisera used for immunohistochemistry were as follow: a 1:12,000 dilution of rabbit anti-human CB5, a 1:40 dilution of mouse anti-human CYP17, a 1:500 dilution of rabbit anti-human HSD3B1, and a 1:500 dilution of rabbit anti-human SULT2A1. These dilutions were based on our prior studies of human and rhesus steroidogenic tissues. We also immunostained some sections for a neuroendocrine marker of the adrenal medulla by use of rabbit anti-human Chromogranin A antiserum (DAKO, Cat # N1535), diluted 1:500 in PBE buffer and incubated as above. After being rinsed in Tris buffer, all sections were incubated with linking and labeling reagents contained in the Multi-Species Ultra Streptavidin Horseradish peroxidase detection kit with DAB as chromogen (Covance). Sections were then counterstained with hematoxylin and coverslipped after dehydration using graded ehanols to xylene.

Digital photography of the slides was accomplished through the use of a Zeiss Axio Imager M2. Images were captured as Tiff files and composite figures were prepared using Power Point software. Magnifications cited in this paper are the product of the eyepiece lens magnification (10×) and those of the objective lenses (2.5, 5, and 10×).

3. Results

Although adrenal sections from all specimens were usually found to contain regions that were immunopositive for HSD3B, CYP17, SULT2A1, and/or CB5, the distribution and abundance of these elements of the steroidogenic pathway varied with morphologic zonation and developmental stage. There were no apparent differences in the patterns or intensity of immunostaining between the adrenals of male and female chimpanzees at similar ages.

3.1. Newborn

In the newborn chimpanzee ($n = 3$), the cortex was found to contain a narrow outer cortical zone (approximately 25% of the total cortical thickness) resembling that of the human that has been termed the neocortex and a broad inner area previously described as the fetal zone (Fig. 1A). The outer portion of the neocortical area in the chimpanzee resembles the zona glomerulosa whereas the inner part of the neocortex appears to be similar to the cell groupings in the human fetal adrenal characterized as the transitional zone (likely precursor to the zona fasiculata). HSD3B staining was mainly restricted to cells in the outer 2/3rds of the neocortex; minimal, if any, immunostaining for this steroidogenic enzyme was noted in the fetal zone (Fig. 1B). CYP17 staining was noted throughout the fetal zone and the inner neocortex but was absent from the outer neocortex (Fig. 1C). The immunostaining for CB5 (Fig. 1D) was found throughout the fetal zone but not in the neocortex. The immunohistochemical staining pattern for SULT2A1 (not shown) was similar to that of CB5 in the adrenals of newborn chimpanzees.

3.2. Early infancy

In the adrenal of a 1 month old chimpanzee, the outer cortex area was broadened compared to that of the newborns and was immunopositive for HSD3B but not the other steroidogenic markers. Immunostaining for SULT2A1 and CB5 persisted in the broad fetal zone, as did that for CYP17, but not in the neocortex.

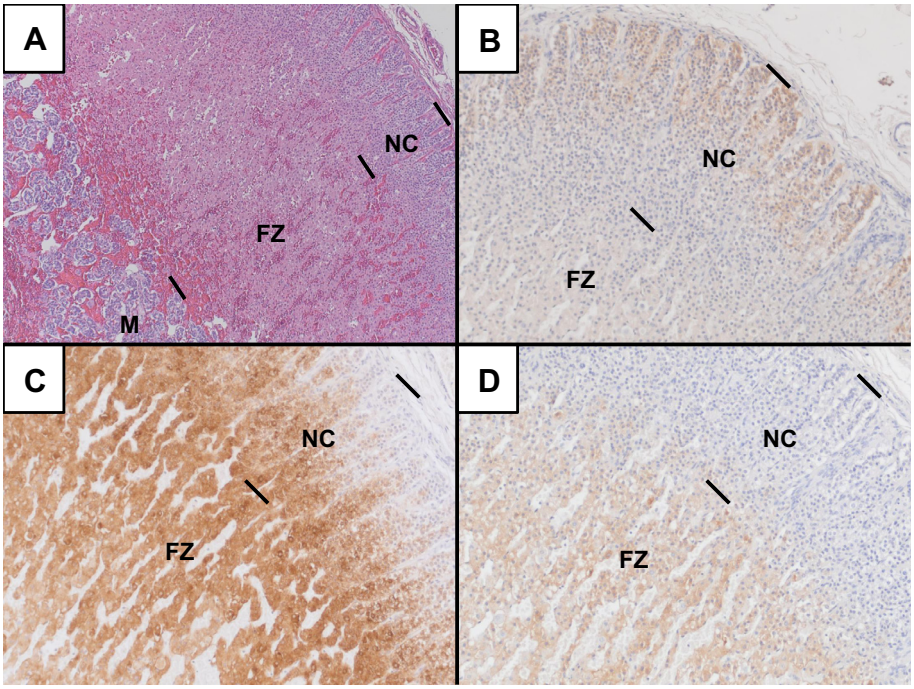

Fig. 1. Adrenal sections from a newborn chimpanzee. The dark lines approximate the borders between the adrenal capsule, the neocortex (NC), the fetal zone (FZ), and the medulla (M). Panel A: Hematoxylin and eosin stained section, 50×. Panel B: HSD3B, 100×. Panel C: CYP17, 100×. Panel D: CB5, 100×.

In the adrenal of a 3 month old chimpanzee, however, there were marked changes compared to that of the newborn. There was evidence for marked regression of the fetal zone and broadening of the outer cortex (Fig. 2A). Coincident with these morphologic changes, there was a widening of the HSD3B positive portion of the cortex (Fig. 2B) and a relative shrinkage in the cortical region expressing SULT2A1 (Fig. 2C) and CB5 (Fig. 2D). Staining for CYP17 (not shown) extended from the innermost cells expressing HSD3B through the innermost area of the cells expressing CB5 and SULT2A1. There also was an amorphous area between the cortex and the medulla (as defined by expression of Chromogranin-A and morphologic appearance by H & E staining), that contained a few scattered clusters of cells that immunostained for CB5, SULT2A1 and CYP17. It was in this perimedullary region in which involution of the fetal zone appeared to be occurring.

The histologic appearance of the area immediately surrounding the medulla at 1 month, 3 months and 13 months is shown in Fig. 3. Compared to that seen in the term fetal adrenal, in the 1 month adrenal (Panels A-1 and A-2), we noted a loss of cortical cells and an increase of acellular material and scattered macrophages containing brown refractile pigment that would be consistent with hemosiderin. We did not notice any such pigment containing cells in the outer cortex. There also was evidence of hemorrhage in the inner aspects of the fetal zone. Many of the cortical cells in this perimedullary region were swollen and vacuolated, consistent with that seen in autophagic cells (Edinger and Thompson, 2004; Gurpinar et al., 2013; Rodrigues et al., 2009). Some of these large cells contained nuclei and in others there were no nuclei visible; however, some nuclei of these cells appeared to be degenerated, but not apoptotic (Gurpinar et al., 2013). Also, in this area were numerous cells with spindle-shaped nuclei, consistent with fibroblast-like cells. Additionally, there were cortical cells that appeared to be apoptotic (Edinger and Thompson, 2004) in the perimedullary region.

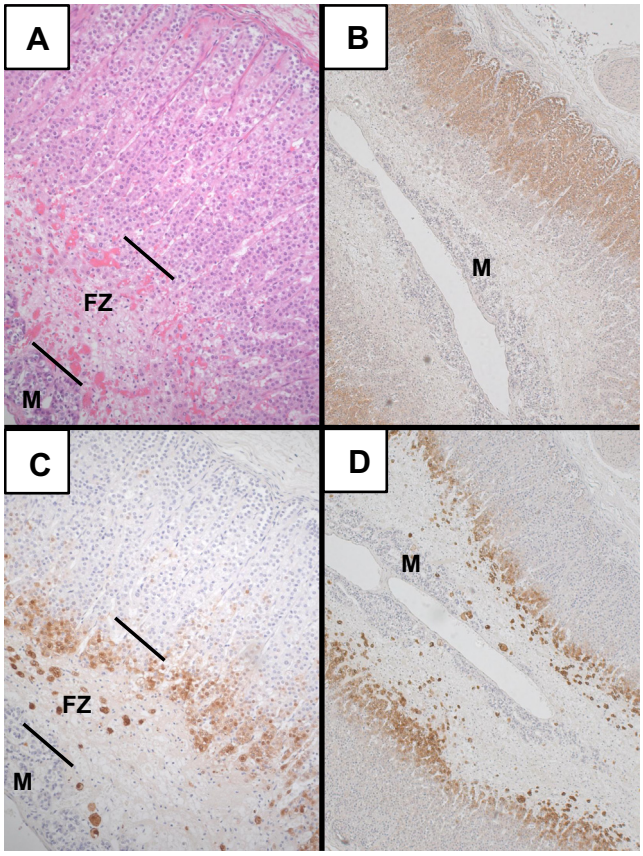

Fig. 2. Adrenal sections from a 3 month old chimpanzee. The dark lines approximate the borders of the regressing fetal zone (FZ); the medulla is indicated by M. Panel A: hematoxylin and eosin stained section, 100×. Panel B: HSD3B, 50×. Panel C: SULT2A1, 100×. Panel D: CB5, 50×.

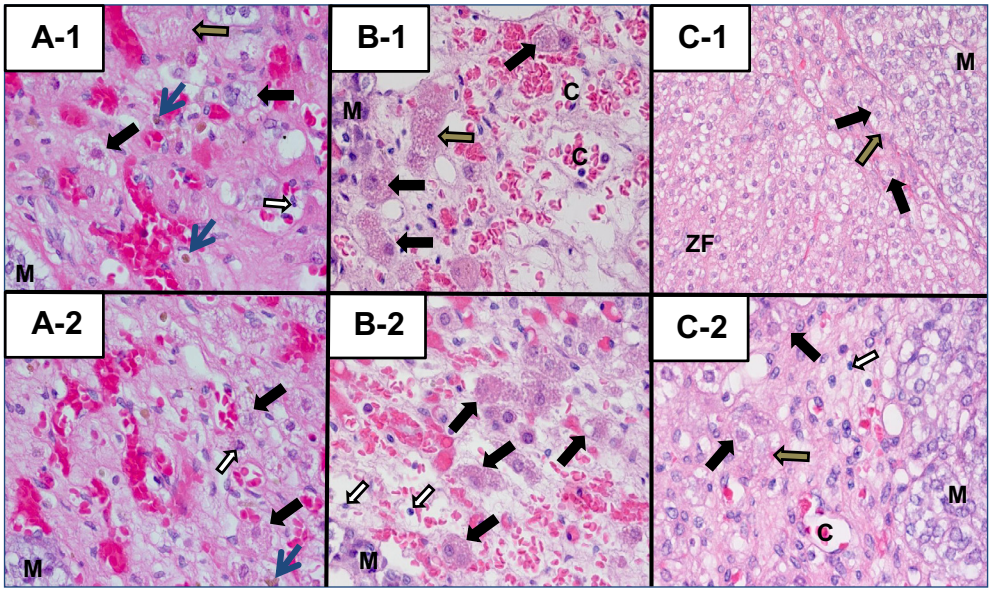

Fig. 3. Morphologic characteristics of chimpanzee fetal zone involution. The medulla is indicated by M, capillaries are indicated by C and the zona fasiculata is indicated by ZF. Hematoxylin and eosin stained sections showing the region of the fetal zone near the medulla are shown for the 1 month (Panels A-1 and A-2, both 400×), 3 month (Panels B-1 and B-2, both 400×) and 13 month (Panels C-1, 200×, and C-2, 400×) infant chimpanzees. Autophagic appearing cortical cells with a nucleus are indicated by solid black arrows and those without nuclei are shown by brown filled arrows; of note, multiple nuclei of the autophagic appearing cells are degenerated, but not apoptotic. Apoptotic appearing cortical cells are indicated by unfilled arrows. Macrophages containing brown pigment, presumably hemosiderin, are indicated by blue arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

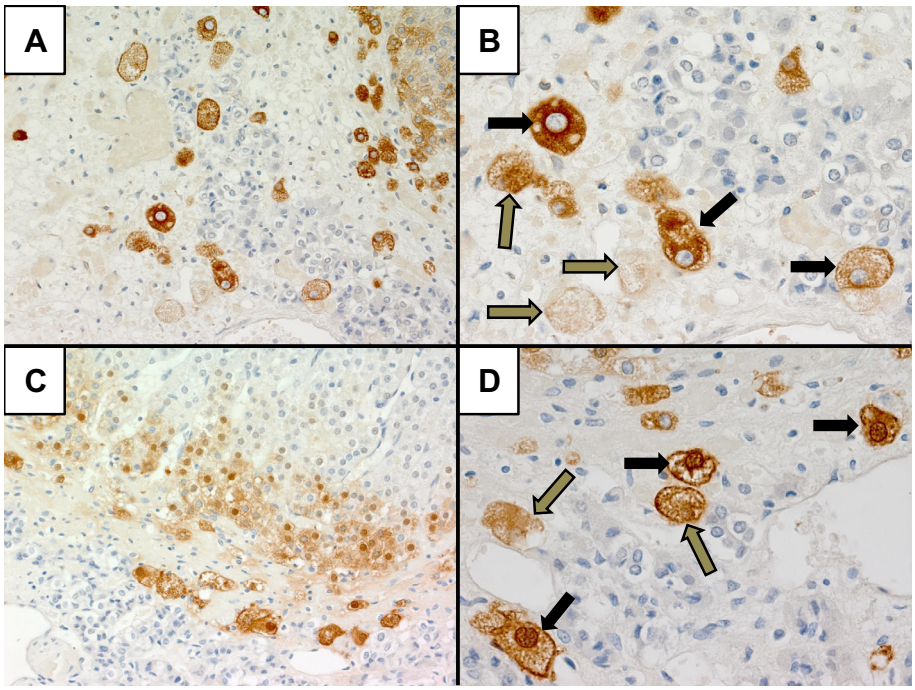

Fig. 4. Immunohistochemical characteristics of cortical cells in the fetal zone of the 3 month old chimpanzee. Panels A and B: CB5 at 200× and 400×, respectively. Panels C and D: SULT2A1 at 200× and 400×, respectively. Autophagic appearing cortical cells with a nucleus are indicated by black arrows and those with no visible nucleus are indicated by brown filled arrows; again, degenerative changes are present in the nuclei of some of these cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In the adrenal of the 3 month chimpanzee, we noted (Fig. 3, Panels B-1 and B-2) large numbers of cortical cells near the medulla that appeared to be autophagic (with and without nuclei visible) as well as cells appearing to be undergoing apoptosis. Autophagic and apoptotic cells were not prominent elsewhere in the adrenal. However, by 13 months of age, there were only a few cells in the perimedullary region that appeared to be autophagic or apoptotic; zona fasiculata-like cells predominated at the cortical-medullary border, which was defined by a thin capsule (Fig. 3, Panels C-1 and C-2).

When we closely examined the immunohistochemical nature of the cells that appeared to be autophagic at 3 months of age, (Fig. 4),

we noted that many such cells were immunopositive for CB5 (Panels A and B) and SULT2A1 (Panels C and D). These cells did not express HSD3B. Some such cells had a prominent nucleus and in others the nucleus was not visible upon focusing though the tissue section. The cells with the least intense immunostaining were usually those in which the nucleus was not seen.

3.3. Later infancy

Further changes were noted in immunohistochemical characteristics of the chimpanzee adrenal adrenal at 13 months of age. Virtually the entire cortex was immunopositive for HSD3B and there was striking immunostaining for CYP17 that extended from the medulla

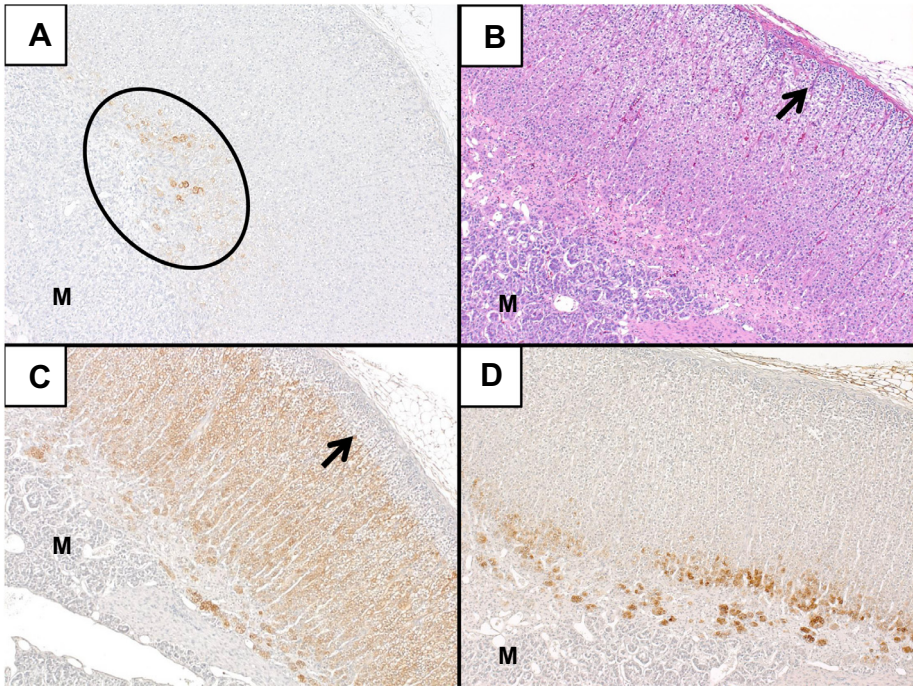

Fig. 5. Adrenal sections from a 13 month old chimpanzee (A) and a 3 years old chimpanzee (B, C, D). Panel A: CB5, 50×. The few scattered immunopositive cells are shown within the circle near the medulla (M). Panel B: Hematoxylin and eosin stained section, 50×. Panel C: CYP17, 50×. Panel D: CB5, 50×. The junction between the zona fasiculata and zona glomerulosa is indicated by the arrows.

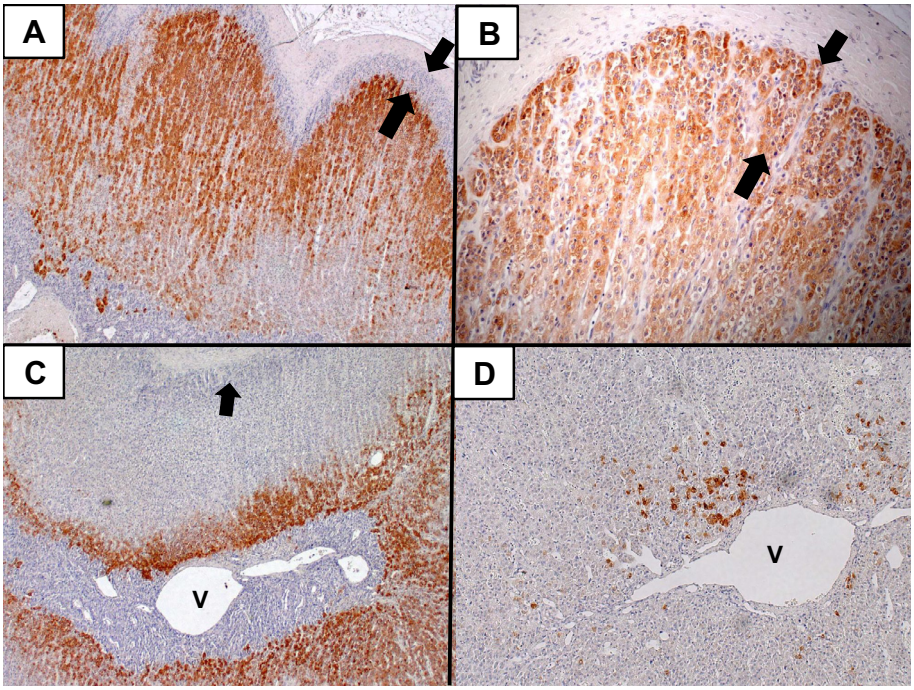

Fig. 6. Adrenal sections from a 12 years old chimpanzee. Panel A: CYP17, 25×. Panel B: HSD3B, 100×. Panel C: CB5, 25×. Panel D: SULT2A1, 50×. The arrows point out the zona glomerulosa. The V indicates the central medullary vein.

to the junction with the cell layers resembling the zona gomerulosa. On the other hand, there were only a few scattered groups of CB5-positive cells in the innermost cortical area adjacent to the medulla (Fig 5A); the staining profile for SULT2A1 (not shown) was similar to that of CB5 but SULT2A1-positive cells were less widespread. The abundance of CB5 and SULT2A1-expressing cells was strikingly diminished in relation to that seen at 3 months.

3.4. Early juvenile

In the adrenal of a 3 years old chimpanzee, as shown in Fig. 5B there was evidence for a distinct zona glomerulosa and zona fasciculata and an inner cortical zone that adjoined an unorganized group of cells surrounding the medulla. HSD3B staining (not shown) was present throughout the zona glomerulosa and zona fasciculata whereas CYP17 was localized to the zona fasciculata and the inner cortical zone (Fig. 5C). CB5 staining (Fig. 5D) was noted in the thin innermost cortex (presumably the zona reticularis and/or residual fetal zone) adjoining the medulla and the abundance of such CB5-positive cells was increased compared to that noted above at 13 months (Fig. 5A). SULT2A1 staining (not shown) also was noted within this inner cortical zone but was detectable in strikingly fewer cells than was CB5.

3.5. Young adult

The adrenal of a young adult chimpanzee (12 years old) was found to have 3 distinct cortical zones. CYP17 staining (Fig. 6A) was noted in the zona fasiculata and zona reticularis but not the outer cortical zone (zona glomerulosa). On the other hand, both the zona glomerulosa and fasiculata were positive for HSD3B (Fig. 6B). In this adrenal, the zona reticularis was considerably broader than in the 3 years old and was immunopositive for CB5 (Fig. 6C) as it was for CYP17 (Fig. 6A); the zona reticularis was not positive for HSD3B (not shown). As had been noted in the 3 years old, SULT2A1 (Fig. 6D) staining of the young adult chimpanzee adrenal was restricted to the zona reticularis and was less widespread in this zone than that for CB5 (Fig. 6C).

3.6. Mature adult

We also noted that cortical cell clusters within the medulla that co-expressed both CYP17 (Fig. 7A) and CB5 (Fig. 7B) were fairly common in the adult chimpanzee adrenal as also has been noted in the human adrenal (Bornstein et al., 1994). Many such clusters of cortical cells in the medulla also expressed SULT2A1 but not HSD3B. Note also that the zona reticularis as defined by CB5 staining (Fig. 7B), although somewhat irregular in its border with the fasiculata, comprised about 1/3 of the cortical width in this 21 years old's adrenal.

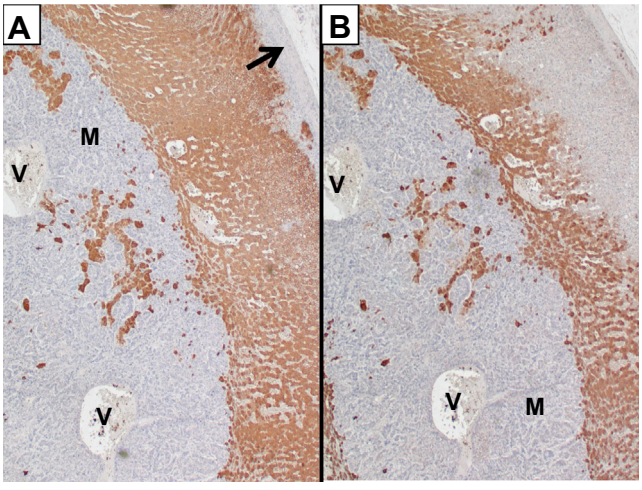

Fig. 7. Adrenal sections from a 21 years old chimpanzee showing intramedullary cortical cells. Panel A: CYP17, 25×. The medulla (M), central vein (V) and adrenal capsule (arrow) are pointed out. Panel B: CB5, 25×.

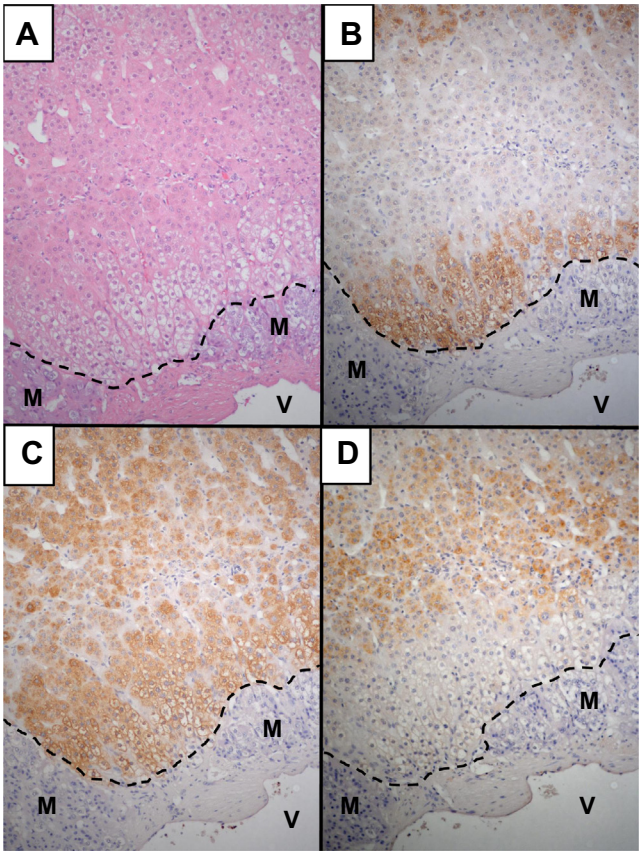

Fig. 8. Adrenal sections from a 31 years old chimpanzee demonstrating the cortical cuff. Panel A: hematoxylin and eosin stained section, 100×. The dashed line in each panel indicates the border between the cortex and the medulla (M) and the central vein (V). Panel B: HSD3B, 100×. Panel C: CYP17, 100×. Panel D: CB5, 100×.

The adult chimpanzee adrenal also was noted to have a cortical cuff around the central adrenal vein (Fig. 8A) (similar to that noted previously in the human) with cells resembling zona fasiculata that expressed HSD3B (Fig. 8B) and CYP17 (Fig. 8C) next to the vein and medulla, and then cells that expressed CYP17 and CB5 but not HSD3B (Fig. 8D) furthest from the vein. Many of the cells that co-expressed CYP17 and CB5 also were immunopositive for SULT2A1 (not shown).

4. Discussion

The development of the human adrenal cortex involves several changes in structure and functional capacity during fetal life and after birth (Lanman, 1961; Mesiano and Jaffe, 1997). During fetal development, the adrenal cortex is composed of a large inner cortical mass known as the fetal zone and a thin outer rim of cells termed the neocortex or definitive zone. At mid-gestation, the fetal adrenal is as large as the fetal kidney and by the end of gestation, the fetal adrenal gland is as large as that of the adult human, with the fetal zone comprising over 70% of the total cortical mass. The human fetal adrenal serves an integral physiologic partner in pregnancy maintenance by virtue of the massive production in the fetal

zone of estrogenic precursors, especially DHEA and DHEA sulfate, which are converted into estrogens by the placenta. Soon after birth, the fetal zone of the human adrenal involutes by a process that likely involves apoptosis (Bech et al., 1969; Bocian-Sobkowska et al., 1998; Spencer et al., 1999) and this regression is accompanied by reductions in circulating levels of DHEA and DHEA sulfate (Forest et al., 1978; Garagorri et al., 2008).

Development of a structurally and functionally similar adrenocortical zone during fetal life has been noted in only a few other mammalian species. While there has been considerable progress in characterizing the development and function of the rhesus and baboon fetal adrenal (Ducsay et al., 1991; Leavitt et al., 1999; McNulty et al., 1981; Nguyen et al., 2008), there is no information concerning the functional zonation of the chimpanzee adrenal cortex. In our study, we found that the adrenal gland of the newborn chimpanzee bears considerable similarity to that of the human adrenal at term in that both have a large inner cortical zone and a thin outer cortex that resembles the zona glomerulosa and a compressed zona fasiculata of the adult. Although we did not perform any quantitative measurements in this study, we believe that the relative thickness of the outer cortex of the newborn chimpanzee appears to be greater than that of the human, as also was shown by Czekala et al., (1983). By virtue of the presence of CYP17, CB5, and SULT2A1 and the absence of HSD3B staining in the fetal zone, we suggest that the fetal zone of the chimpanzee has the same steroidogenic capacity as the human fetal zone to produce DHEA and DHEA sulfate, which could serve as precursors for placental estrogen formation in pregnancy of the chimpanzee. It is known that estrogen production in chimpanzee pregnancy follows a temporal pattern that is similar to that of the human (Reyes et al., 1975; Smith et al., 1999) Fetal adrenal androgen production also is evident in other primates such as the rhesus and baboon and likely arises from the fetal zone as the result of the prominence of CYP17 and CB5 and a paucity of HSD3B (Mesiano et al., 1993; Leavitt et al., 1999; Mapes et al., 2002) and, at least in the rhesus, the presence also of SULT2A1 (Parker et al., 2000b).

The outer cortical zone of the newborn chimpanzee adrenal was found to express HSD3B but not CYP17, CB5 or SULT2A1, which is the same profile that we and others have found in the human neocortex and which is similar to the expected enzymology of the zona glomerulosa (Barker et al., 1995; Dharia et al., 2004; Dupont et al., 1990; Mesiano et al., 1993; Parker et al., 1994, 1995; Suzuki et al., 2000). In the chimpanzee, we also noted the presence of cortical cells located between the glomerulosa-like outer cortex and the fetal zone that were arranged in columns and contained HSD3B and CYP17 but little, if any, CB5 or SULT2A1. Such a cortical arrangement in the newborn chimpanzee is similar to the pattern that characterizes the 'transitional' zone of the human fetal adrenal and the zona fasiculata of the adult human adrenal. Although we are unable to document the fetal developmental changes in the chimpanzee adrenal, it is likely that the fetal zone requires trophic support of the hypothalamic–pituitary axis for its growth and steroidogenic output, as has been shown to be the case for the fetal adrenal of the human and several other primates (Lanman, 1961; Mesiano and Jaffe, 1997).

During infancy in the chimpanzee, we found evidence for adrenocortical reorganization that qualitatively resembles that noted for the human, with involution of the fetal zone along with development of a medullary capsule and expansion of the outer cortex into zona glomerulosa and zona fasiculata (Bech et al., 1969; Bocian-Sobkowska et al., 1998; Dhom, 1973; Sucheston and Cannon, 1968). There was persistence of a broad inner cortex that was immunopositive for CB5 and SULT2A1 in a 1 month old chimpanzee, but by 3 months of age, there was clear evidence for involution of this zone. Subsequently, there were only a few scattered cells remaining in the inner cortical area that were immunopositive for CB5 and SULT2A1 in the adrenal of a 13 month old chimpanzee; the rest of the cortex in this specimen displayed the characteristic staining pattern of the zona fasiculata (HSD3B and CYP17 positive) and zona glomerulosa (HSD3B positive, CYP17 negative).

The majority of the loss of the human fetal zone occurs within the first few weeks of life, followed by a much slower process occurring over the first 1–2 years of life (Bech et al., 1969; Bocian-Sobkowska et al., 1998; Sucheston and Cannon, 1968). The process of regression of the human adrenal has been described as an initial hemorrhagic process (Bech et al., 1969) that is associated with striking levels of apoptosis (Bocian-Sobkowska et al., 1998; Spencer et al., 1999). Our results are suggestive that apoptosis and autophagy (Edinger and Thompson, 2004; Gurpinar et al., 2013; Rodrigues et al., 2009;) may play a role in regression of the fetal zone in the chimpanzee. There also is some indication of hemorrhage in the chimpanzee fetal zone. Our sample size is too limited to provide more than an approximation of the timeline involved in reorganization of the adrenal cortex during infancy. Nevertheless, it appears that fetal zone regression is largely complete within the first year of life and development of the zona reticularis occurs later.

There also is regression of the fetal zone during early infancy in other related species such as the rhesus monkey, baboon, and marmoset monkey (Levine et al., 1982; McNulty et al., 1981; Nguyen et al., 2008). In the baboon and rhesus, however, the regression of the fetal zone appears to coincide with the formation of the zona reticularis during infancy. In both species, a group of small densely staining cells have been noted between the outer cortex and the fetal zone that expands as the fetal zone shrinks (Ducsay et al., 1991; McNulty et al., 1981). Ducsay et al. considered the dense zone of the baboon to represent an area of degeneration of fetal zone cells; this aspect of baboon adrenal development does not appear to have been explored further. On the other hand, the dense band in the neonatal rhesus adrenal was believed to be the site of transition into zona fasiculata cells in the outer dense band and into reticularis cells in the inner portion of the dense band (McNulty et al., 1981), and immunohistochemical studies of the infant rhesus adrenal (Nguyen et al., 2008) may be consistent with such an interpretation. We did not notice the presence of a dense band of cortical cells in the newborn or infant adrenals of the chimpanzee and have not seen any reference to this type of morphologic feature in the human adrenal during the perinatal period.

By 3 years of age, the chimpanzee adrenal was found to have a thin, but continuous inner cortical zone that was positive for both CB5 and CYP17 and to a lesser extent, SULT2A1; the cells of this zone were negative for HSD3B. This staining pattern is characteristic of that seen in the zona reticularis of the human adrenal cortex that emerges during adrenarche and persists thereafter (Endoh et al., 1996; Dharia et al., 2004; Kennerson et al., 1983; Suzuki et al., 2000). A similar staining profile also is characteristic of the rhesus zona reticularis (Mapes et al., 1999).

The presence of a zona reticularis that expresses a phenotype consistent with the capacity for DHEA and DHEA sulfate synthesis in the young chimpanzee and in older specimens regardless of gender, in which the zone became broader though young adulthood provides the functional basis for the developmental changes in circulating levels of adrenal androgens that has been documented during adrenarche and thereafter in the chimpanzee (Bernstein et al., 2012; Collins et al., 1981; Copeland et al., 1985; Cutler et al., 1978). The development of the zona reticularis in the chimpanzee differs somewhat from that in the rhesus and marmoset monkey and the baboon. In the rhesus and the baboon, the zona reticularis appears to develop as the fetal zone is regressing (Ducsay et al., 1991; McNulty et al., 1981; Ngyuen et al., 2008), thus masking developmental changes in adrenal androgen production that would be indices of the process of adrenarche (Castracane

et al., 1981; Nguyen et al., 2008). On the other hand, the zona reticularis of the marmoset monkey is only apparent in females and the enzymology and androgen production is subject to regulation by social status and ovarian function (Pattison et al., 2005, 2007).

With aging in the human, there is maintenance of corticosteroid secretion but impairment in DHEA and DHEA sulfate secretion by the adrenal (Dharia and Parker, 2004; Parker et al., 2000a). There also is evidence for decreased adrenal androgen production during aging in the rhesus and baboon (Lane et al., 1997; Sapolsky et al., 1993). We have noted that there appears to be narrowing of the zona reticularis but not the zona fasiculata/glomerulosa in the human adrenal during aging (Dharia et al., 2005; Parker et al., 1997) that may contribute to the striking adrenal androgen deficiency seen in humans during aging. Recently, Blevins et al., 2013 have provided evidence for gradual reductions in circulating levels of DHEA sulfate in the aging chimpanzee; these changes, however, are not as striking as that noted in the human. Whether such reductions in the chimpanzee, (or in the rhesus and baboon) during aging can be attributed to morphologic changes in the adrenal will require further study.

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