Perinatal Ontogeny of Brain Growth in the Domestic Pig (44469)

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Abstract. **The perinatal development of the brain is highlighted by a growth spurt whose timing varies among species. The growth of the porcine cerebrum was investigated from the third trimester of gestation (70 days postconception) through the first 3.5 weeks of postnatal life (140 days postconception). The shape of the growth curves for cerebrum weight, total protein mass, total cell number (estimated by DNA content), and myelination (estimated by cholesterol accretion) were described. The growth velocity of cerebrum weight had two peaks, one at 90 days and the other at 130 days postconception, whereas that of total protein was greatest from 90 to 130 days postconception, and that of total DNA was greatest between 90 and 110 days and again at 130 days postconception. The growth velocity for total cholesterol continued to increase during the entire period, suggesting that myelination continued after the growth spurts for cells (protein and DNA). The growth velocity patterns observed in these contemporary pigs suggest that this species may be an appropriate model for human brain development, not only in the perinatal pattern of increase in mass of the cerebrum, as established previously, but also with regard to the patterns of cellular development and myelination.** [P.S.E.B.M. 2000, Vol 223]

Knowledge of the ontogeny of normal growth of the central nervous system is important in understanding the effects of nutrition of the fetus and neonate on later physical and mental development. Prenatal and early postnatal malnutrition is known to affect behavior and mental development (1–5). Dobbing *et al*. (6–8) emphasized the importance of this stage of brain growth on the response to nutritional insults and noted differences among mammalian species in the timing of the brain "growth spurt," which they defined as that transient period of growth when the brain is growing most rapidly. Dobbing and Sands (9) and Dobbing (8) concluded that the pig is an appropriate model for human infants because its brain growth spurt, like that of the human, extends from late prenatal to early postnatal life. This is in contrast to other species, such as the rat whose brain growth spurt is postnatal or the guinea pig whose growth spurt is prenatal.

Late fetal and early postnatal life are periods when the offspring is especially vulnerable because it has a relatively high growth rate and, hence, increased nutritional needs, which must be met by the mother. Therefore, the growth of the offspring at this chronobiological age is vulnerable to factors that directly affect its growth capacity, as well as factors that impact the capacity of the mother to provide it with sufficient nutrition.

Mammalian brain growth is characterized by increases in neuroneal cell number to the adult level before the major phase of glial cell multiplication begins. Brain growth during the growth spurt consists primarily of glial cell multiplication, dendritic growth, and synaptic connectivity followed by rapid myelination (8). Oligodendroglial cells synthesize myelin and form myelin sheaths from their cell membranes (10, 11). Thus, two important measurements with which to assess brain growth are cell number and myelination.

We report here on the growth of the cerebrum in con-

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temporary crossbred domestic swine from the third trimester of prenatal life (70 days postconception) through the first 3.5 weeks of postnatal life (140 days postconception). The purpose of this work is to extend the findings of Dickerson and Dobbing (6) to contemporary pigs and to describe the shape of the growth curve of the pig cerebrum with respect to total organ weight, total protein mass, total cell number (estimated by DNA content), and myelination (estimated by cholesterol accretion). The data may be useful in defining the vulnerable periods of long-term functional development in response to nutritional insults during perinatal life.

Materials and Methods

Animals. A total of 15 contemporary crossbred multiparous sows were euthanized at 70, 80, 90, 100, or 110 days of gestation (three sows at each stage of gestation). All fetuses were removed from each uterine horn within 5 min after electrical stunning and exsanguination. Body weight, whole brain weight (combined weights of cerebrum, cerebellum, and medulla oblongata), cerebrum weight, and cerebellum weight of all (171 total) live fetuses were recorded. Cerebrum from two randomly selected males and two randomly selected female fetuses within each of three litters at each sampling time ($n = 12$ fetuses per sampling time) was frozen in liquid nitrogen and stored at −70°C for protein, DNA, and cholesterol analyses. Suckling-age crossbred pigs (same genetic background as fetuses) from three litters (two females and two males from each litter, $n = 12$ per sampling time) were sacrificed by intramuscular injection of an overdose of ketamine/acepromazine and exsanguination at 120 days ($n = 12$), 130 days ($n = 12$), and 140 days ($n = 12$) postconception, corresponding to about 5, 15, and 25 days postnatal age, respectively. Body weight, whole brain weight, cerebrum weight, and cerebellum weight were recorded, and cerebrum was frozen as indicated above.

Tissue Processing. Cerebra from 60 fetuses and 36 postnatal pigs were removed from storage at −70°C, crushed mechanically, pulverized with a mortar and pestle, and divided into two aliquots for analysis (one for protein and DNA assay and the other for cholesterol assay).

Protein and DNA Assays. Weighed aliquots of powdered cerebrum were homogenized at 4°C in chilled, deionized water. The protein concentration of the homogenate was determined colorimetrically (12), with human serum albumin used as a standard, and human plasma samples (Monitrol-ES Level 1 Chemistry Control, Baxter Healthcare Corp., Dade Division, Miami, FL) used as controls. An interassay variation of ± 2.5% is tolerated for standard, control, and experimental samples. An intra-assay variation of ± 2.5% is tolerated for control samples. Greater variation mandates repetition of the analysis. The DNA concentration of the homogenate was measured fluorometrically (13) with Hoechst reagent 33258 (Sigma, St. Louis, MO); calf thymus DNA (Type I; Sigma, St. Louis, MO) was used as standard.

Cholesterol Assay. Cholesterol concentration was determined on the saponified lipid extract (14) of cerebrum using a modified (15) colorimetric assay (16). An interassay variation of ± 5% is tolerated for standard experimental samples. Greater variation mandates repetition of the analysis.

Statistical Analyses. Main effects of sex and age and sex × age interaction were tested by analysis of variance with repeated measures (17) [sources of variation were: sex, degrees of freedom (d.f.) = 1; age, d.f. = 7; sex × age, d.f. = 7; error, d.f. = 79]. Overall means and SD at each sampling time are presented graphically for each trait. Incremental growth of cerebrum and cerebellum and incremental accretion of cholesterol, protein, and DNA in cerebrum at each sampling interval, expressed as a change from the previous 10-day measurement for each trait, were calculated to describe the growth velocity curve for each trait.

Results

Data on body weight and weight of whole brain, cerebrum, and cerebellum of all 171 live fetuses and of 60 fetuses (30 males and 30 females) randomly selected within litter and 36 piglets (18 males and 18 females) are summarized in Table I. Males and females did not differ significantly in any trait measured; therefore, we report only the combined data for all 171 fetuses and for the 96 selected fetuses/piglets. As expected, means for randomly selected fetuses compared closely with means of the entire population.

Growth curves for body weight, cerebrum weight, and cerebellum weight from 70 to 140 days postconception are shown in Figures 1, 2, and 3, respectively, for the 60 selected fetuses. The brain growth spurt [defined by Dobbing (8) as "that transient period of growth when the brain is growing most rapidly"] is portrayed as a velocity curve superimposed on the respective weight growth curves for cerebrum and cerebellum in Figures 2 and 3. Body weight from 70 to 140 days postconception (Table I and Fig. 1) increased curvilinearly, representing the period preceding the steep inflection characterized in the sigmoid growth curve of all biological growth systems. Body weight at 140 days postconception represented only about 2% of adult body weight of 300 kg (18). Cerebrum weight (Fig. 2) increased from 70 to 140 days, but growth velocity was highest at 90 days (middle of the third trimester of gestation), then declined to 120 days (early postnatal), increased at Day 130 (middle of a 4-week suckling period), then declined to a level similar to that at Day 80, suggesting a biphasic pattern of cerebrum growth velocity in the pig: one peak in late gestation and one in early postnatal life. Cerebrum weight at 140 days postconception was ≈ 40% of that reported in the adult pig [94 g, Pond *et al*. (19)]. Cerebellum weight (Fig. 3), like that of the cerebrum, increased steadily from 70 to 140 days, but incremental growth suggested a different cerebellum growth pattern than that of cerebrum. That is, velocity of cerebellum growth increased sharply from Day 80 through Day 110, then declined to Days 120 and 130 and increased to its highest level at Day 140, when

Table I. Body, Whole Brain, Cerebrum and Cerebellum Weights of Fetuses (70–110 d) and Piglets (120–140 d) Sampled at 10-day Intervals from 70 to 140 Days Postconception

Trait	Age (d)	All live fetuses			Randomly selected fetuses/piglets[a] (Two males, two females/litter)		
		Mean	SD	*n*	Mean	SD	*n*
Body weight (g)	70	213	45[b]	38[c]	212	58	12[d]
	80	300	88	45	289	127	12
	90	537	120	34	547	165	12
	100	859	152	30	884	189	12
	110	823	167	24	790	210	12
	120				2125	472	12
	130				3963	537	12
	140				6496	1670	12
Whole brain weight (g)	70	5.93	0.50		5.83	0.74	
	80	10.87	1.42		10.73	2.04	
	90	18.91	1.41		18.48	1.46	
	100	25.61	1.71		24.73	1.81	
	110	30.85	3.19		31.11	2.83	
	120				34.51	1.72	
	130				42.08	1.83	
	140				46.11	8.97	
Cerebrum weight (g)	70	5.25	0.53		5.27	0.72	
	80	9.49	1.20		9.23	1.69	
	90	17.04	1.33		16.78	1.48	
	100	22.53	1.66		21.78	1.72	
	110	26.23	2.75		26.51	2.38	
	120				29.32	2.36	
	130				35.08	2.90	
	140				38.96	7.48	
Cerebellum weight (g)	70	0.38	0.17		0.36	0.19	
	80	0.49	0.15		0.49	0.20	
	90	1.01	0.17		1.02	0.20	
	100	2.14	0.32		2.02	0.40	
	110	3.14	0.47		3.21	0.46	
	120				3.83	0.30	
	130				4.41	0.31	
	140				5.67	1.18	

[a] Means for males and females did not differ ($P < 0.05$); therefore, data for males and females were combined.
[b] Mean +/− standard deviation.
[c] Number of animals per group is the same for all traits within each age group.
[d] Number of animals per group is the same for all traits within each age group.

its weight was about 40% of adult cerebellum weight [14 g, Pond *et al*. (19)], as in the case of the cerebrum.

Concentrations of cholesterol, protein, and DNA in the cerebrum at 70–140 days postconception are shown in Table II. Cholesterol concentration remained relatively constant through Day 100, then increased steadily to Day 140. Protein concentration followed a pattern similar to that of cholesterol, except that the increase was apparent at Day 100 and continued to Day 130. In contrast, DNA concentration was highest at Day 70, then declined at Day 80, and remained relatively constant to Day 140. The concentration of a particular structural constituent of an organ is determined by the combined effects of its rates of synthesis, degradation, import, and export in relation to the combined concurrent mass of all other constituents in the organ. Therefore, the observed increases in concentrations of cholesterol and protein in the cerebrum during its rapid growth reflect a greater rate of net accretion of these two constituents than that of other constituents. Because DNA is an index of cell number in an organ, it is apparent that cell multiplication was less than that of protein accretion throughout the period from 70 to 140 days postconception. The failure of DNA concentration to decline during the period of steady growth of the cerebrum after 80 days indicated that cell multiplication (probably glial cells) continued throughout the period from 80 to 140 days. Cessation of hyperplasia would have been associated with a continued decline in DNA concentration as organ size increased between 80 and 140 days.

Accretion curves for total protein, DNA, and cholesterol in the cerebrum from Day 70 to Day 140 postconception (calculated as the product of organ mass × concentration of the respective constituent at each age), are shown in Figures 4, 5, and 6, respectively. Superimposed on each respective figure are growth velocity curves for protein, DNA, and cholesterol.

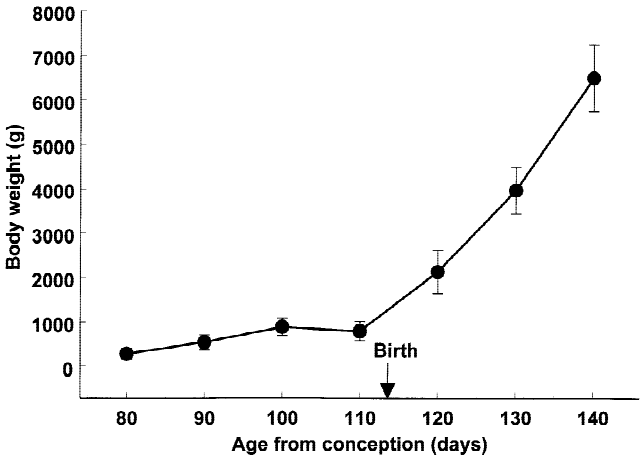

Figure 1. Body weight in pigs from 70 to 140 days postconception (n = 12/age group). Absolute values are represented by solid lines and solid circles (standard deviations are indicated by a vertical bar at each circle); velocity curves are represented by dashed lines and solid triangles.

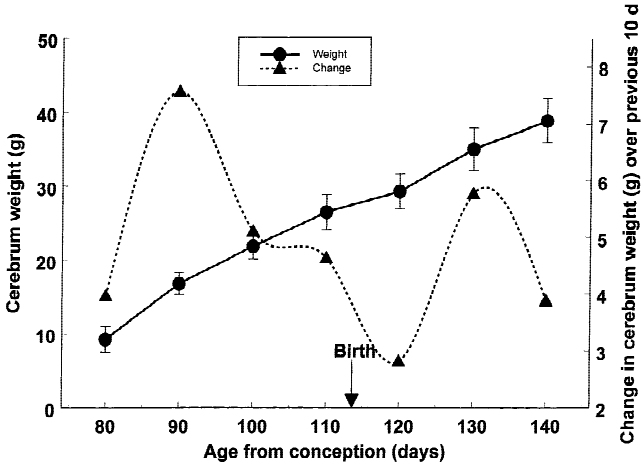

Figure 2. Cerebrum weight and cerebrum growth velocity in pigs from 70 to 140 days postconception (n = 12/age group). Absolute values are represented by solid lines and solid circles (standard deviations are indicated by a vertical bar at each circle); velocity curves are represented by dashed lines and solid triangles.

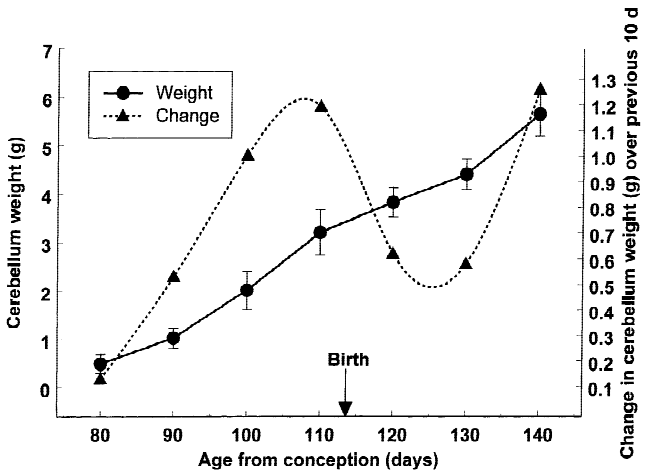

Figure 3. Cerebellum weight and cerebellum growth velocity in pigs from 70 to 140 days postconception (n = 12/age group). Absolute values are represented by solid lines and solid circles (standard deviations are indicated by a vertical bar at each circle); velocity curves are represented by dashed lines and solid triangles.

Table II. Concentrations of Cholesterol, Protein, and DNA in Cerebrum of Pigs from 70 Through 140 d Postconception (n = 12 at each age)

Age (d)	Cholesterol (mg/100 g)		Protein (mg/g)		DNA (mg/g)	
	Mean	SD[a]	Mean	SD	Mean	SD
70	790	216	45.18	3.89	1.97	0.12
80	879	187	43.17	2.82	1.47	0.09
90	761	156	48.88	2.23	1.27	0.05
100	770	272	59.62	2.96	1.37	0.08
110	1119	184	66.67	2.24	1.47	0.07
120	1378	153	78.76	3.69	1.48	0.07
130	1641	229	81.16	3.05	1.45	0.08
140	2202	499	82.91	2.50	1.37	0.06

[a] SD = standard deviation.

Total protein (which includes that present in neuroblasts, neurons, glial cells, and other structural components) increased from 70 to 140 days (Fig. 4), but the protein accretion growth spurt was highest from 90 to 130 days, then declined at 140 days. Cerebrum total protein content at 140 days was ≈ 45% of that of the adult pig [7.2 g, Pond *et al.* (19)].

Total DNA increased steadily from 70 to 140 days (Fig. 5). The growth velocity curve of DNA accretion (Fig. 5) showed a rapid rise from 80 to 90 days, then a more gradual rise to a peak at 110 days, followed by a sharp decline to 120 days, a rise to 130 days, and a second decline to near the 80-day level at 140 days, suggesting a biphasic incremental growth curve somewhat comparable to that observed for cerebrum weight. Total DNA at 140 days was 53.4 mg, similar to that reported in mature sows (19), suggesting that cellularity of cerebrum is at or near the mature level at 140 days in the pig. Neuroneal tissue multiplication precedes that of glial cells in mammalian brain development (8). Although the cellular type represented by total DNA measurement of cerebrum was not determined, the biphasic nature of the growth velocity curve may correspond to an early peak (100–110 days) representing the neuroneal growth spurt followed by a later peak (130 days) representing the glial growth spurt. The reduced velocity of DNA accretion at 140 days and the attainment by 140 days of a cerebrum total cell number similar to that of the adult pig reflect the high degree of maturity of both neuroneal and glial cellularity in the early postnatal life of the pig. Total cholesterol, an index of myelination, increased curvilinearly from 70 days to 140 days (Fig. 6). The rapid rate of cholesterol accretion at the older ages is also evident by the shape of the growth velocity curve (Fig. 6). The persistent accretion of cholesterol and the continued rise in velocity throughout the period from 120 to 140 days, in contrast to the shape of the velocity curves for protein and DNA (both of which were

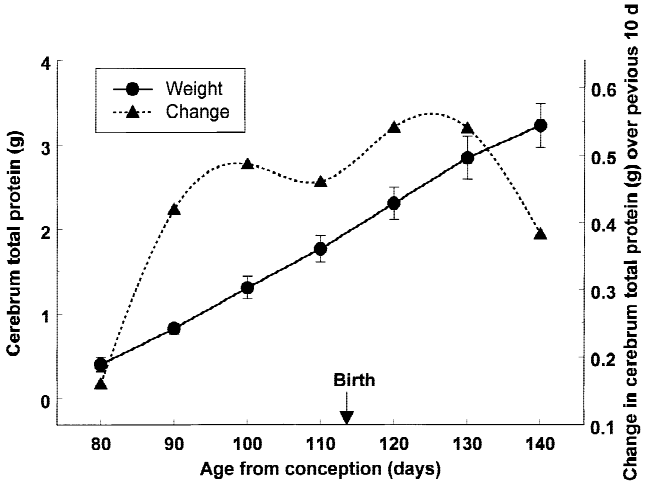

Figure 4. Cerebrum total protein and protein accretion velocity in pigs from 70 to 140 days postconception (n = 12/age group). Absolute values are represented by solid lines and solid circles (standard deviations are indicated by a vertical bar at each circle); velocity curves are represented by dashed lines and solid triangles.

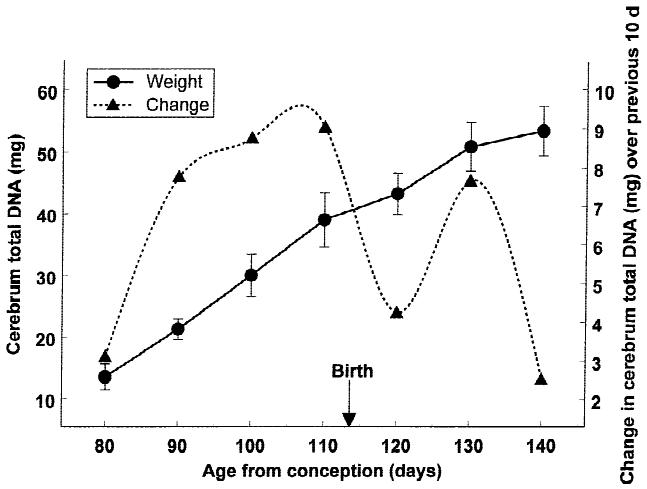

Figure 5. Cerebrum total DNA and DNA accretion velocity in pigs from 70 to 140 days postconception (n = 12/age group). Absolute values are represented by solid lines and solid circles (standard deviations are indicated by a vertical bar at each circle); velocity curves are represented by dashed lines and solid triangles.

indicative of a perinatal growth spurt), strongly suggest increased myelination of cerebrum after the neuroneal and glial growth spurts have peaked, in agreement with data reported in humans (8, 9). Total cerebrum cholesterol at 140 days was ≈ 35% of that reported in adult pigs [2200 mg, Lu *et al*. (20)]. This observation agrees with that in humans, in whom there is a rapid increase in forebrain cholesterol to about 4 years of age, followed by a gradual rise to adult levels (9).

Discussion

Growth of the mammalian brain is characterized by a growth spurt (that transient period of growth when the brain is growing most rapidly), whose temporal relationship to

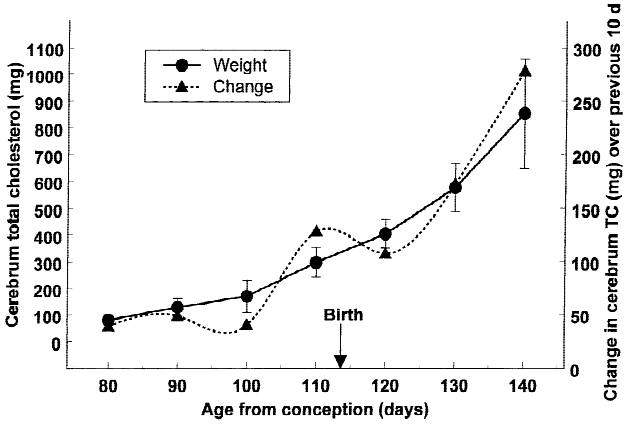

Figure 6. Cerebrum total cholesterol and cholesterol accretion velocity in pigs from 70 to 140 days postconception (n = 12/age group). Absolute values are represented by solid lines and solid circles (standard deviations are indicated by a vertical bar at each circle); velocity curves are represented by dashed lines and solid triangles.

birth of the animal varies greatly among species (8). The velocity of human brain growth is greatest from a few weeks before to a few weeks after birth, whereas that of rat brain is greatest 3 weeks postnatally and that of guinea pig brain is greatest about 3 weeks prenatally (8, 21, 22). Dickerson and Dobbing (6) concluded that the velocity curve of pig brain growth corresponds more closely than that of other species to the velocity curve of the human brain. Our data extend the findings of Dickerson and Dobbing (6) to contemporary pigs, and provide evidence in support of the concept of specific velocity curves for growth of cerebrum and cerebellum and for accretion of protein, DNA, and cholesterol in cerebrum. In mammals, the attainment of adult brain neuron cell number is well under way before the major phase of glial multiplication begins (8). The present data reveal, in the pig, a major growth spurt for cerebrum at 90 days, followed by a lesser, but distinctive spurt at 130 days, the former representing prenatal and the latter postnatal growth. In contrast, the first growth spurt of the cerebellum occurred at 110 days, followed by a second spurt at 140 days. Although this biphasic growth spurt for both organs was not observed by Dickerson and Dobbing (6), who used fewer animals and fewer time points, our observation does support their general concept of a period of rapid brain growth extending from late prenatal to early postnatal life. The growth spurt for cerebrum DNA generally paralleled that of cerebrum weight in that both had a prenatal and a postnatal peak. Whether the early peak in the pig primarily represents neuroneal cell multiplication, and the later peak primarily glial cell multiplication, is not clear. If neuroneal cell multiplication is nearly complete before the major cerebrum growth spurt, as proposed by Dobbing (8), the observed biphasic DNA growth spurt may represent the normal ontogeny of glial cell multiplication in the pig. The shape of the velocity curve for cerebrum protein indicates that the protein growth spurt extends over the entire period from 80 days to 140 days in the pig.

In agreement with Dickerson and Dobbing (6), the growth spurt for DNA and protein was succeeded by an extended period of cholesterol accretion, an index of myelination. There was no evidence of a reduced velocity of cholesterol accretion during the period from 70 to 140 days; in fact, the rapid rise from 120 to 140 days suggested the beginning of a renewed cholesterol growth spurt. Cholesterol is an essential constituent of all animal cells, and is found primarily in membranes. The brain has the highest cholesterol concentration of any organ in the body because of the extensive cell membrane content of myelin. Oligodendroglial cells synthesize myelin and form myelin sheaths from their cell membranes (10, 11). The complex temporal relationships involved in orchestrating the normal neuronal and glial cell multiplication, dendritic growth, synaptic connectivity, and myelination associated with normal brain maturation in the pig are not well understood. For example, Jarvinen *et al*. (23) found increased size of stellate cells in the visual cortex in brains from 8-week-old pigs compared with newborns, but a reduction in total number of dendritic branches from layer IV stellate cells in the auditory and somatosensory cortex in the 8-week-old pigs. Our data define normal growth patterns of pig brain associated with specific schedules of growth velocity for cell multiplication, protein, and cholesterol accretion. The information suggests specific periods of vulnerability to nutritional and other metabolic insults in fetuses and neonates, and the crucial importance of an adequate supply of cholesterol at these times, provided by either endogenous synthesis in the central nervous system or perhaps diet.

Dietary cholesterol supplementation of neonatal pigs increases cerebrum cholesterol content at 8 weeks of age (24) and young adulthood (23) and enhances exploratory behavior (24). These observations suggest that dietary cholesterol either indirectly affects cerebral cholesterol synthesis, or is a direct source of cholesterol to be incorporated into cerebral membranes. Glial cell multiplication, dendritic growth, and synaptic connectivity followed by rapid myelination are associated with the period of most rapid brain growth (8). Our data suggest a prenatal accretion spurt for cerebrum protein (90–100 days) and DNA (100–110 days), followed by a second peak during early postnatal life (120–130 days for protein and 130 days for DNA). Cerebrum cholesterol accretion, in contrast, continues to increase in velocity from 100 to 140 days, with a steady concomitant increase in cerebrum weight and cholesterol concentration. This supports the concept that myelination lags behind cell multiplication, as in humans, and persists at a high rate after the rates of protein and DNA accretion have decreased. Functional associations with the observed velocity curves for cerebrum protein, DNA, and cholesterol accretion are needed to assess the biological importance of these characteristics of the ontogeny of brain development.

Brain development may be vulnerable to nutritional insults imposed during either the late prenatal or early postnatal period of accelerated protein or DNA accretion. Indeed, results of research with neonatal pigs (25) suggest that developmental environment can alter brain morphology, including dendritic branching, during the interval of ontogeny when primary cortical neurons are still undergoing maturation. Brain myelination may be more vulnerable to nutritional insult in the neonate than in the fetus, in which the cholesterol accretion rate is lower. Therefore, the pig is likely to be a useful animal model for evaluating the effects of dietary and other insults during various phases of the perinatal period on human brain growth and development.

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