Differential Sensitivity of Children and Adults to Chemical Toxicity

Biological Basis

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Children, particularly neonates, can be biologically more sensitive to the same toxicant exposure on a body weight basis than adults. Current understanding of the rates of maturation of metabolic capability and evidence from case examples on pharmaceuticals, drugs of abuse, environmental contaminants, and dietary and endogenous agents indicate that human infants up to approximately 6 months of age are typically—but not always—more sensitive to chemical toxicity than adults. For most chemicals, the immaturity of infant biotransformation, elimination, and other physiologic systems usually produces higher blood levels for longer periods. There is metabolic capacity for most tested substances in the newborn, although it is quite low and immature for some chemicals. For some chemicals, unique metabolic pathways not available in the adult human can also be utilized by the newborn. The newborn's metabolic capacity rapidly matures and, by about 6 months of age, children are usually not more sensitive to chemical toxicity than adults. By then, most metabolic systems are reasonably mature, becoming almost completely capable by 1 year of age. In many cases children are less sensitive than adults. Whether children are at greater risk from chemical exposures is another question. Risk depends on both inherent sensitivity and exposure conditions. If chemical exposure levels remain below those capable of overwhelming a child's metabolic detoxification systems and producing toxicity, children will be at no greater risk than are adults. Children of all ages are still developing so even if they are exposed to chemicals at levels below those of adults, they may be at greater risk than adults. However, as long as those exposure levels are still below those required to produce toxicity, children will not be at greater risk. © 2002 Elsevier Science (USA)

INTRODUCTION

Increasing attention over the past 10 years has been given to the potentially disproportionate impact that environmental chemical exposures[1] might have on the health of infants, children, and the developing fetus. That concern led to the children's health provisions of the 1996 Food Quality Protection Act, to President Clinton's 1997 Executive Order *Protection of Children from Environmental Health Risks and Safety Risks*, to establishment of the U.S. Environmental Protection Agency's (EPA's) Office of Children's Health Protection and Children's Health Protection Advisory Committee, and to numerous other children's health research and policy efforts.

Much of the current concern surrounding children's health and risks from chemicals in the environment is attributed to the 1993 National Academy of Sciences (NAS) report *Pesticides in the Diets of Infants and Children*. That report concluded that children may experience quantitatively and qualitatively different exposures to chemicals than do adults, that children may be more or less sensitive to chemically induced toxicity compared to adults, and that standard approaches to risk assessment and regulation may not always account explicitly for potential age-related differences in exposure and toxicity. The report raised concerns that, at least in some cases, children may not be protected adequately by current regulatory policies.

Response to the NAS report has included vigorous debate about the extent to which children may or may not be experiencing disproportionate impacts from chemical exposures. In many cases, scientific data are lacking, fostering increased reliance on inference and concomitant tension among stakeholder viewpoints. Physiologic and pharmacologic differences due to age have become better understood, however, as have age-related differences in chemical exposure patterns and levels. Age-related effects on susceptibility appear to

[1] The term "environmental chemical" is used to refer to substances of industrial, natural, agricultural, or pharmaceutical origin to which exposure may occur orally, dermally, via inhalation, or across the placenta, either intentionally or unintentionally.

depend on the chemical of concern, the effect that is observed, the dose that is received, its duration, and the period of development during which exposure occurred. Infants, children, or the developing fetus are more sensitive than adults in some cases and less sensitive in others.

Nonetheless, controversy over whether the young are inherently more sensitive to chemical toxicity than adults continues. There are many physiologic and pharmacologic reasons why the sensitivity of children and adults to chemical exposures may differ. The developing organism experiences many complex, integrated events involving the regulation of cell growth, differentiation, and morphogenesis. Interference with those events through mutation or through altered cell division, hormone activity, enzyme function, or energy sources can have significant adverse impacts on development. Many environmental factors can have an impact on normal development, including nutritional adequacy (e.g., protein, vitamin, and folic acid availability), maternal smoking and alcohol consumption, prescription drugs, and chemical contaminants such as lead and organic mercury.

The relevant question, however, is not whether children are inherently more sensitive than adults but whether they are at greater risk. This question requires the integration of information about chemical hazards and exposure and evaluation of regulatory agency approaches to setting limits on chemical exposures. Some are concerned that the current regulatory scheme permits chemical exposures that entail risks to neonates, infants, or children because inadequate attention is given to their potentially greater sensitivity to chemical toxicity. This is a reasonable concern and one that can be addressed by examining in some detail how and why neonates, infants, and children can be inherently more sensitive to toxicity than adults.

GROWTH AND DEVELOPMENT

Evaluating chemical toxicity to developing systems is of major importance because damage to a physiological system prior to its full development can permanently affect the system (NAS, 1993). Although not as dramatic as the growth from conception to birth, human postnatal growth during the first year of life is extraordinary: a typical human infant increases in weight by about 200% and in length by 50% (Sparks, 1998). The rates of growth of the various organs in the human infant are not identical to the overall rate of growth. Figure 1 illustrates the rate of growth for several organs in humans. The differences in the rates of growth have toxicological implications, notably with respect to "windows of vulnerability."

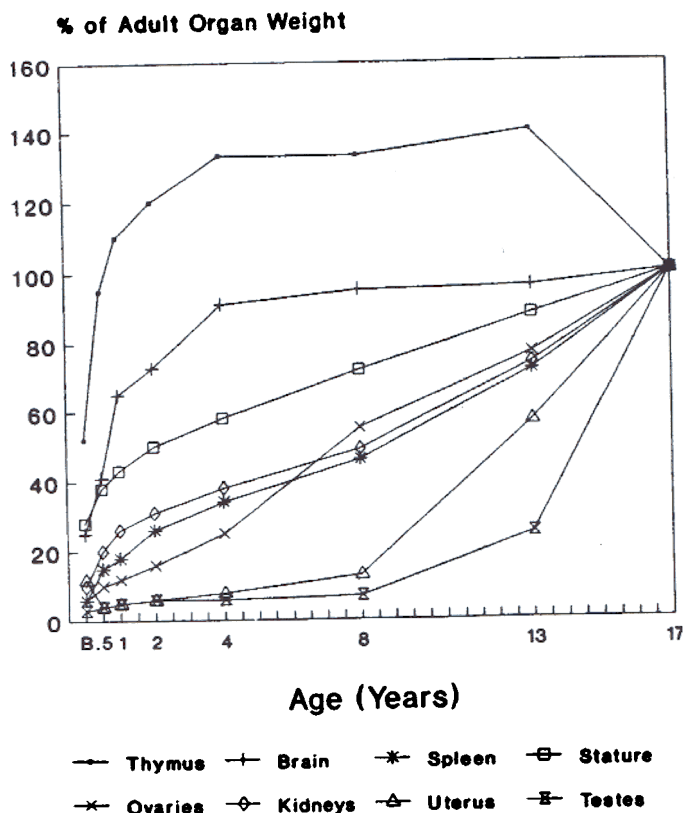Sensitivity during development makes children unique because only a *developing* human or animal can

FIG. 1. Organ development and stature or body weight as a percentage of adult values by age in the human. Source: Based on data from Altman and Dittmer, 1962.

experience toxicity that is integral to the developmental process. The young can also experience acute or chronic toxicities that are not necessarily unique to the young, but that may occur at a lower dose due to the immaturity of defense mechanisms that are fully developed in the adult. In this sense too, the young may be more sensitive than adults. The differences in response between the young and the adult are sometimes discussed in terms of either pharmacokinetics or pharmacodynamics, the former considering the factors producing an effective dose and the latter considering intrinsic organ or system susceptibility. Both, of course, are important to every toxic response, but when the young and the adult experience the same dose, the special vulnerability of the young becomes evident. For example, many substances that can be used with relative safety by the pregnant mother can be quite harmful to her developing child. These include alcohol, tobacco, cocaine, opioids, amphetamines, and other psychotherapeutic drugs (Kopecky and Koren, 1998).

The period of developmental vulnerability for the young starts at conception and extends through gestation, parturition, infanthood, childhood, and up to and through adolescence. Although most vulnerable during

the earlier periods, children are still developing during adolescence. Because of the developmental processes, acute or chronic chemical exposure has the potential to result in serious anomalies that may persist or develop further at a later age. A dramatic example of this is the incidence of vaginal cancers produced in the daughters of women exposed to therapeutic doses of diethylstilbestrol from the mid-1940s to 1970 (Herbst et al., 1971).

That the young are not totally without pre- or postnatal defenses to exogenous chemicals is quite clear, however. As the authors of *Pesticides in the Diets of Infants and Children* (NAS, 1993) noted, infants and young children are remarkably robust to the effects of therapeutic treatment with drugs despite their often greater sensitivity. It is also evident that many, if not most, enzymatic mechanisms capable of metabolizing exogenous chemicals mature to adult capacity within 6 to 12 months after birth. Whether or not an agent's toxicity is of a developmental nature, an adequate dose is still required to produce an adverse effect. If the young are not exposed to a toxic dose, a greater intrinsic susceptibility will not have consequences to their health or normal development. As this analysis stresses, the relevant question is not children's greater sensitivity to chemical agents but whether, given their chemical environment and often greater susceptibility, they are at greater *risk*.

A major objective of this review is to describe how exogenous agents may interfere with the normal process of development to produce abnormal cells, tissues, organs, and function. We briefly review below, with some examples and with reference to some of the relevant science, how and when the young may be more vulnerable to exogenous chemical exposures.

Periods of Development

Many complex biological changes occur during development that can have profound consequences on sensitivity to the effects of exogenous chemicals. The outline below is incomplete, but is meant to illustrate one oft-made but still important point: the developing fetus, newborn, infant, and child are not simply miniature adults.

Gestation averages 266 days, or $9\frac{1}{2}$ months in humans, counted from the day of fertilization, and is commonly divided into three periods: preembryonic, embryonic, and fetal. The first 2 weeks after fertilization is the preembryonic period, characterized by cell division of the zygote, formation of the solid ball of cells called the *morula*, and formation of the hollow blastula and gastrula stages, all prior to the building of organ systems. Throughout these early stages, the conceptus is relatively insensitive to the action of agents (teratogens) that can cause congenital malformations later (Kaplan and Bolender, 1998).

The embryonic period extends from the 2nd week to the 8th week after fertilization. During this period the initial morphogenesis of the organ systems begins and, in many cases, ends. It is during this morphogenic period, when organs are being formed, that the embryo is most sensitive to malformations. Hematopoiesis (blood cell formation) begins in the yolk sac during the 3rd week of development and progenitor cells migrate to the fetal liver by 5 to 8 weeks; the liver expands to about 10% of the body weight by the beginning of fetal life (Hamilton and Mossman, 1972).

The fetal period (from 8 weeks after fertilization until birth) is characterized by rapid growth in weight and size of the conceptus, reflecting the growth of individual organs. During the first trimester, increased serum levels of cortisol and thyroxine appear to promote the early partial expression of numerous enzyme systems, including those related to glycogen synthesis and amino acid metabolism (Greengard, 1977). During the fetal period the conceptus becomes increasingly resistant to the action of teratogens. This does not mean that some organ systems cannot become malformed during fetal life. An example is the brain, which continues both physical and functional development through the fetal period and beyond (Dobbing, 1976). The brain's extended period of development extends its susceptibility; for example, the cognitive deficits in children produced by low levels of lead exposure are more closely associated with postnatal rather than prenatal lead exposure (Goyer and Clarkson, 2001). More congenital defects occur in the brain than any other organ but most of these are neural tube defects, which occur in the first trimester (Conner and Ferguson-Smith, 1984). Other organ systems continue morphogenesis during the fetal period (e.g., palate, ear, and external genitalia) but, in contrast to the brain, most other organ systems complete morphogenesis by birth. All organs expand and undergo differentiation of their cell populations, but the pattern of development can vary from organ to organ. It is important to remember that the establishment of the gross form of the organ does not necessarily mean the establishment of function. For example, while the human fetal gastrointestinal tube at 6 months closely resembles that of the newborn infant, the development of enzyme systems necessary for digestion continues through birth and well beyond (Gregus and Klaassen, 1998).

The perinatal period includes the time shortly before and after birth. Parturition creates a new situation for the offspring with respect to many physiological systems. Changes in heart rate, peripheral vascular resistance, and a redistribution of blood flow occur. At birth elimination of substances across the placenta ceases and, with the collapse of the placental circulation, hepatic blood flow and oxygen supply dramatically decrease. The new condition has several implications for the elimination of compounds. For example,

elimination of transplacentally acquired drugs slows considerably. Soon after birth, the plasma concentration of bile acid and bilirubin rises several-fold as placental elimination ends and neonatal hepatic function is temporarily impaired due to a diminished oxygen supply. An increase in glomerular filtration rate (GFR) begins immediately after birth as the kidney assumes its clearance and regulatory functions. The ability of the kidney to concentrate urine to adult levels is not reached until after birth. There is a decline in total body water content during fetal life and major changes in its distribution. Total body water declines from 95% during the early fetal period to reach 80% at 8 months of gestation and 75% water at term. The percentage of water that is extracellular sharply decreases during the perinatal period while intercellular water gradually increases (Brace, 1998).

Breathing begins in fetal life but is intermittent and is not involved in gas exchange; it becomes continuous at birth (Rigatto, 1998). At 28 weeks of gestation, the human lung has the potential for the support of gas exchange, although unassisted ventilation in unlikely because the lung and its surfactant system are still underdeveloped. Immediately after birth the lung must function as the organ of gas exchange and, in a matter of minutes, dramatic events must occur to convert the fluid-filled newborn lung to one that is gas-filled. Fluid absorption and surfactant production are two major challenges for the newborn alveolar and bronchiolar epithelium. The immediate postnatal period taxes the oxygen transport system more than any other period of life. The high oxygen affinity of fetal blood is well adapted to oxygen uptake in the placenta but has disadvantages in early postnatal life. The newborn needs more oxygen than the fetus; the oxygen consumption of most species increases by 100–150% in the first few days of life (Avery, 1974). To meet the oxygen demand, rapid increases occur both in oxygen affinity and in oxygen-carrying capacity. On the first day of life, the P_{50} in normal infants is 19.4 ± 1.8 mm Hg, in contrast to 27.0 ± 1.1 mm Hg for the normal adult. P_{50}continues to increase gradually and reaches normal adult values at 4–6 months of life (Delivoria-Papadopoulos and McGowan, 1998).

Glucose is the major energy source for the human fetus and approximately 70% of the fetal glucose absorbed is converted to fat (VanAerde et al., 1998). For most mammalian species, birth represents an abrupt transition from a low-fat to a high-fat diet (VanAerde et al., 1998). At birth the maternal supply of glucose ceases abruptly and the newborn must rapidly mobilize liver glycogen stores (glycogenolysis) to meet its metabolic needs until it receives a new source of glucose from milk. Within 12 h after delivery glycogen stores are exhausted and the newborn must be capable of producing glucose from the triglycerides in milk

(gluconeogenesis). Cytosolic phosphoenolpyruvate carboxykinase, the rate-limiting enzyme for gluconeogenesis, is low at birth (0–25% of adult values) but increases within hours. Endocrine changes that occur at birth, particularly the surge of plasma glucogon and the fall in insulin, favor mobilization of lipids from peripheral tissues. Shortly after birth there is a sharp rise in plasma concentrations of free fatty acids (FFA) (by a factor of 3 within 30 min after birth) (Roux and Romney, 1967). Significant amounts of FFA can "spill over" from the primary binding site in albumin to secondary sites and displace bound drugs. Consequently the free fraction of many drugs, such as valproate, salicylate, and diazepam, rises sharply in neonatal blood after birth (Nau and Plonait, 1998). Unlike the newborn rat, the newborn infant has a high body fat content (16%) and can sustain a period of starvation without hypoglycemia (Narkewitz and Girard, 1998).

The infant period, 1–12 months after birth, is characterized by continued changes in pharmacokinetic and pharmacodynamic characteristics, many of which are described in more detail below in the section Pharmacokinetics and Development. For example, in the weeks following birth, a decrease in renal resistance associated with a rise in arterial blood pressure contributes to a rise in renal blood flow (Robillard et al., 1981). Serum levels of bile acids, elevated at birth, gradually decline to adult levels by 6–12 months of life.

During the infant period, breast feeding is strongly recommended by pediatric physicians and has been shown to decrease the risk of gastrointestinal and respiratory disease in infancy (Rieder, 1998) and to increase IQ (Lucas et al., 1994). At least 50% of mothers breast feed their children and over 90% of new mothers take medication for treatment of acute or chronic illness (Matheson, 1985). The potential for maternal medication through breast milk is a concern. In most cases, the passage of drugs from the maternal circulation is governed by passive diffusion of the free (nonprotein-bound) nonionized form. The majority of drugs and environmental chemicals with a molecular size smaller than 200 Daltons can cross from maternal plasma into breast milk (Rieder, 1998). While the amount of drug available from breast milk is typically less than 1% of the maternal dose and is usually of negligible significance, a small number of drugs are contraindicated during lactation. These include antimetabolites, iodine-containing chemicals, psychoactive drugs, and recreational chemicals (Rieder, 1998). Another potential concern is the tendency of persistent lipophilic environmental contaminants to accumulate in breast milk and cause high levels of intake in breast-fed infants (van der Molen et al., 1996).

An infant's daily intake of dioxin through breast feeding, for example, can far exceed any comparable intake from food. Most authorities consider that any risks from

the accumulation of TCDD by this means are more than outweighed by the health and psychological benefits of breast feeding (WHO, 1988).

Genetics and Signaling as Regulators of Development

Normal growth and development are controlled by large networks of regulatory genes and occur as a progression of states of spatially defined regulatory gene expression (Davidson et al., 2002). This progression leads to specification, or the process by which cells in each region of the developing animal come to express a given set of genes. The spatial cues that trigger the expression of specific sets of genes as different tissues develop are generally provided by signaling ligands produced by other cells as a consequence of their own prior states of specification. Signaling ligands such as semaphorins trigger gene expression during development by interacting with their receptors to provide "intercellular cross-talk" during organogenesis in order to regulate activities as diverse as axonal guidance, lymphocyte activation, control of vascular endothelial cell motility, and lung branching morphogenesis (Goshima et al., 2002). A cell's decision to self-renew, differentiate, or remain quiescent is dependent on an integration of multiple signaling pathways as well as on cell density, metabolic state, ligand availability, type and levels of receptor expression, and downstream cross-talk between distinct signaling pathways (Sommer and Rao, 2002).

In contrast to physiological transcriptional responses, which can vary in response to stimuli such as changes in the levels of nutrients or introduction of toxicants or pathogens and then return to normal, developmental transcriptional systems always move inexorably forward, never reversing direction. This property is a consequence of the complex gene regulatory network that is active during development (Davidson et al., 2002) and has two possible results: perturbations of critical components of the network during development may have consequences that cannot be repaired as the system continues to move forward; and the complexity of the system may lend it plasticity that permits compensation for perturbations, should they occur.

Susceptibility to the developmental effects of environmental agents has been shown to be modulated by genetic variability in growth factor regulators and homeobox genes (Faustman et al., 2000). For example, an elevated risk of cleft palate has been reported for infants of mothers who smoke and carry an uncommon allele for transforming growth factor α (Shaw et al., 1996). An increased risk of birth defects in smoking mothers has also been associated with a polymorphism in the homeobox genes (MSX) responsible for vertebrate limb development (Hwang et al., 1998).

The Developing Nervous System

Particular attention has focused recently on developmental neurotoxicity, partly in response to the EPA's new developmental neurotoxicity testing guidelines and partly due to concerns about potential subtle neurotoxicologic effects in children due to low-dose chemical exposures. As an example of how organ development can affect its susceptibility to damage, this section briefly describes the development of the nervous system.

Soon after division of the fertilized ovum, the two blastomeres begin the process of neural development, a progressive specialization of cells governed by both genetic factors and extrinsic influences (Pomeroy and Segal, 1998). The basic processes involved in the development of the nervous system include cellular replication, migration, differentiation, myelination, and synapse formation (Anthony et al., 2001). Transformation of the embryonic cells into neural epithelium is complex and varies in different regions. By day 16 of gestation, the segregation of germ layers occurs and cuboidal ectoderm is transformed into columnar neural epithelium and a thickened neural plate. This primary neurulation gives rise to the neural plate and subsequently to the neural tube and the cells of the neural crest. By day 18 of gestation a midline groove forms in the neural plate, initiating the folding and fusion of the neural plate into the neural tube. The fusion process begins on day 22 of gestation and by day 26, both the anterior and the caudal ends of the neuropore are closed (Sidman and Rakic, 1982). Fusion of the neural folds is one of the best known examples of the critical role that apoptosis, or programmed cell death, plays in development; the cell's ability to regulate apopostosis directly affects its sensitivity to developmental interference from toxicants (Mirkes, 2002). As the neural tube closes, cells at the edge of the neural plate separate from the neural epithelium and migrate into the extracellular matrix to become neural crest cells. Neural crest cells migrate widely to become neurons and glia in dorsal root and autonomic ganglia. Neural crest cells retain a relatively broad developmental potential as they begin migration and their ultimate fate is strongly influenced by local factors (Pomeroy and Segal, 1998). By 35 days of gestation, rudiments of the cerebrum and cerebellum are evident and cerebral vesicles begin to form. By 6–8 weeks of gestation, along the neuroaxis, clusters of neurons begin to form nuclei of the brain stem, thalamus, and hypothalamus. Elements of the spinal cord arise by a different process (secondary neurulation), utilizing an undifferentiated group of cells near the caudal ends of the neural tube. A process of canalization occurs during the ensuing weeks, forming the caudal end of the spinal cord.

By 6–8 weeks of gestation, the basic structure of the central nervous system is formed, concluding embryogenesis. Following the embryonic period, expansion of the cerebral hemispheres occurs by the proliferation of neurons and glia and then by the growth of neural and glial processes. Over the first 3 months of the postembryonic period, the full adult complement of neurons proliferates and populates the developing brain (Pomeroy and Segal, 1998). Neuronal proliferation ends at midgestation (about embryonic day 125). Glial proliferation, in contrast, continues throughout life (Prival, 1975). Prior to gestation day 40, when the cells in the neural epithelium are increasing geometrically, appropriately timed insults would be expected to have a larger impact than those occurring after day 40 (Pomeroy and Segal, 1998). As neurons migrate along radial glia, they extend processes that are soon recognizable as axons. By 20 weeks of fetal life, the shape of the corpus callosum has roughly assumed that of the adult (Rakic and Yaklovlev, 1968). Migrating neurons begin to sprout dendrites as soon as they arrive at the cortical plate. Dendritic length of prefrontal cortex increases as much as 5- to 10-fold in the first 6 months of life and then continues at a slower rate (Schade and van Groenigen, 1961). Synapses appear in the human cerebral cortex as early as the third month of gestation (Molliver et al., 1973). Synapse number in the frontal cortex can increase for up to 2 years after birth (Huttenlocher, 1979). Over the course of months to years, a refinement of these synaptic connections occurs and the axons become ensheathed in myelin. Myelination exhibits pronounced regional and temporal variation. The earliest myelin is detected within motor nerves by the sixth fetal month, but the myelogenic cycle can be detected in cortical processes beyond the third decade of life (Yakovlev and Lecours, 1967). The continuing development of the nervous system may explain the remarkable sensitivity of babies and young children to global insults of the nervous system, such as that resulting from brain tumor irradiation therapy, and the unusual ability of children to recover from focal injury to the central nervous system (Radcliff et al., 1992). Cortical dentritic and synaptic development is abundant and seemingly malleable in the young, providing a degree of "plasticity," or ability to recover from injury, that is not attainable by mature brain (Stiles, 1995).

The blood–brain barrier. Most brain capillaries offer much greater resistance to the passive diffusion of polar molecules than most other capillaries. The diffusional resistance is primarily the result of tight junctions between endothelial cells, the absence of pores within the cells, and a thicker, more developed basement membrane surrounding each cell (Reese and Karnovsky, 1967). The transporter protein P-glycoprotein plays an important role in the brain barrier function by removing substances such as drugs from the cell membrane and cytoplasm. In rats and mice, capillary diffusion

decreases by a factor of 30 during the 3rd and 4th postnatal weeks (Bar and Wolff, 1972). In humans, however, P-glycoprotein is expressed in the fetal brain at adult levels by the third trimester (van Kalken et al., 1992). Because of sparse human data and significant species differences, the timing of barrier growth and development in prenatal and postnatal humans is still uncertain (Laterra, 1998).

Defects in brain development. Defects in brain development can occur during all phases of gestation and postnatal development: primary neurulation and neural tube closure (3–6 weeks gestation), neuronal proliferation (2–10 months postnatal), neuronal migration (3–10 months postnatal), axon outgrowth (birth–3 months postnatal), dendritic growth and synapse formation (6 months–1 year postnatal), and myelination and synaptic rearrangement (birth–years postnatal). The timing of the teratogenic event is critical. In general, the most severe anomalies occur early in gestation. For example, total failure of neural tube closure (craniorachischisis) originates no later than day 22 of gestation, failure of anterior neural tube closure (anencephaly) not before day 25, and failure of posterior neural tube closure (myeloschisis) no later than day 26. Anencephaly arises from errors of anterior neural tube closure. Although the cause of most neural tube defects is unknown, recent studies suggest a model of multigenetic inheritance with environmental influence (Gilbert et al., 1986). The maternal ingestion of some antiepileptic drugs, especially valproate, can also increase the incidence of these malformations (Nau et al., 1991). Examination of the teratogenic effects of aminopterin, a folic acid antagonist, led to the discovery that folic acid supplementation around the time of conception can significantly reduce the occurrence of neural tube defects (Smithells et al., 1981; Czeizel and Dudas, 1992). Later in gestation, aberrant organization of the cortex and of axon myelination leads to disorders of cerebral cortical function, which in the least affected cases may account for learning disorders or other subtle neurologic dysfunction.

PHARMACOKINETICS AND DEVELOPMENT

Complex changes occur in the body's abilities to absorb, distribute, metabolize, and eliminate substances during development, both before and after birth. Those abilities develop at different rates, so the body may respond to chemical challenges in different ways at different ages. For example, the filtering function of the kidney develops faster than its absorptive or secretory functions, so the body's ability to eliminate substances that are partially reabsorbed or secreted is diminished for a longer period than for those requiring only filtration. The nature and extent of absorption, distribution, metabolism, and elimination are determined by

TABLE 1
Biochemical and Physiological Changes in Adults and Infants

Absorption	
Gastric pH	Neutral at birth, pH <4 within 2–4 days, and gradually decreases to adult values
Pepsin	Diminished at birth (3–13%), adult level by 3rd month of life
Lipase	Diminished at birth (3–22%), adult level by 6th month of life
Trypsin	Diminished at birth (13–30%), adult level by 3rd month of life
α-Amylase	Diminished at birth (0.2–3%), adult level by 2–3 years
Gastric acid secretion	Lower in young infants, increases to adult level in 3 months
Gastric emptying time	Lower in neonates, may reach adult level at 6–8 months
GI motility	Slow and irregular motility in infants
GI microflora	Rapidily colonized after birth; high in neonates
Distribution	
Extracellular water	40% body weight in neonates, 26–30% in 1 year, 50–60% in adults
Total body fat	Proportionately higher in infants
Total plasma protein	Lower in neonates/infants than in children/adults and children, composition different in neonates/infants
Volume of distribution	Tends to be reflect relatively larger water compartment; adult levels of transport protein present at birth
Metabolism	
Phase 1	Pronounced individual variation
Phase 2	Glucuronidation deficient at birth, reaches adult level by 3–4 weeks, sulfation active in neonates and young children
Biotransformation	Alternate pathways in neonates, rates are lower in neonates
Elimination	
Glomerular filtration rate	Low in neonates, increases rapidly during first year
Renal tubular secretion	Matures later than glomerular filtration; reaches adult level by 1 year
Renal function and clearance	Greater in older infants and young children than in older children/adults
Body water	
Total body water	90% bw at second trimester, ~75% at birth, decreases to 60% (adult value) by 3 months
Extracellular	60% bw at second trimester, 40–45% at birth, decreases to ~30% by 1 year
Intracellular	Increases early in gestation to ~35% bw by second trimester, remains near 40% throughout infancy
Plasma water	Unchanged during development 4–5% bw range
Body fat	
Total body fat	Increases from 1–3% bw in midgestation to ~16% at term and peaks at ~25% by 6–9 months postnatal; decreases as a percentage of body weight over 6–7 years of childhood to adult values

Note. Data adapted from Radde (1985), Reed (1997), Blumer and Reed (1992), Cohen (1987), Kauffman (1992a), Nagourney and Aranda 1998), Sparks (1998), Chemtob (1998), Costarino and Brans (1998), and Brace (1998).

the underlying growth rates and functional integration of these organ systems. This section summarizes many of the biochemical and physiological changes that can have an impact on the body's sensitivity to chemical toxicity. Those changes are also summarized in Table 1.

Absorption

Gastrointestinal absorption of xenobiotics overall does not appear to change dramatically with age but several factors that affect absorption do change. Gastric secretion is low in newborns and gastric pH is correspondingly high (6–8 compared to 1.5 in adults). The higher pH can result in decreased absorption of weak acids and increased absorption of weak bases. Gastric emptying is also prolonged, peristalsis is irregular, and intestinal motility is reduced in the newborn. In comparison to the adult, newborns also exhibit differences in their intestinal flora, which have been shown to affect the absorption of vitamin K (Gustaffson, 1962). The presence of metabolic enzymes such as P450 3A and efflux transporters such as P-glycoprotein in the epithelium of the small intestine plays a significant role in

reducing the oral bioavailability of drugs (Suzuki and Sugiyama, 2001); their rates of maturation are likely to influence absorption. Children often appear to absorb drugs as completely and sometimes more completely than adults (Rowland and Tozer, 1980).

Bile acids play an essential role in the digestion and absorption of dietary lipids. The primary bile acids, cholic acid and chenodeoxycholic acid, are synthesized from cholesterol in the liver. Bile acid metabolism and turnover are not fully developed at birth; primary bile salts exhibit a transient elevation in the first few weeks and then decline steadily for several years while liver function matures (Heubi et al., 1982). As a result, the absorption of fats and lipid-soluble substances may be affected.

With the exception of lipase, digestive enzymes are generally present at birth at lower activities than in adults (see Table 1), although enzyme activity increases rapidly during the first year of life. Glucose absorption in infants is 3–4 times less efficient than in adults (Koldovsky, 1978). The digestion of cow's milk protein increases by 80% during the first 4 postnatal months of life (Koldovsky, 1978). There is evidence from both human and animal studies that the immature intestine

can allow the passage of intact macromolecules, including immunoglobulins, β-lactoglobulin, and bovine serum albumin (Grand et al., 1976). The serum of infants contains a higher percentage of antibodies to food antigens than does the serum of adults, suggesting that food proteins are absorbed intact in sufficient quantity to elicit an immunologic response (Rothberg, 1969).

The skin of the neonate is thinner than that of infants and adults. In extremely preterm infants, there is almost no stratum corneum and the skin is fragile and permeable. At birth, regardless of gestational age, the skin rapidly cornifies over a period of 2–3 weeks, providing an effective epidermal barrier to chemical agents (Evans and Rutter, 1986).

During the final prenatal period of fetal lung development in humans, important maturational processes occur, including the production of surfactant necessary to decrease surface tension at the air–liquid interface of the alveoli (Bolt et al., 2001). Lung alveolar surface area is greater than that of adults on a body weight basis, which, together with children's higher ventilation rates, contributes to children's often greater absorption through inhalation. The number of alveoli continues to increase until about age 8, after which they increase in size instead. Lung growth continues throughout childhood into early adulthood, reaching a plateau and then declining with increasing age (Burri, 1977).

Distribution

The distribution of a drug is influenced by several factors, including the size of the body water and lipid compartments, regional blood flow, presence of transport proteins, and the degree to which drugs bind to plasma and tissue proteins. High protein binding tends to limit the drug or chemical to the vascular space. In the preterm newborn, both albumin and α-acid glycoprotein concentrations and binding affinities are low compared to adults (Nau et al., 1998). These proteins primarily bind acidic and basic substances, respectively, and their reduced levels in preterm newborns result in an increased fraction of free drug and in distribution of free drug outside the vascular compartment.

Infants have a higher percentage of water in lean body tissues than adults. The additional water is primarily extracellular, so that the extracellular water compartment in infants is about twice that of adults (Widdowson and Dickerson, 1964). Together, decreased protein binding and increased extracellular fluid volume in neonates result in a greater volume of distribution for relatively polar chemicals. For example, newborns need twice as long as adults to eliminate lidocaine because of the large distribution volume that must be cleared of the drug (Morselli et al., 1980). This example also illustrates the difficulty in predicting the effects of drugs in the newborn; age-related differences in distribution and excretion can nullify each other, so that the

pharmacologic potency of a drug can be comparable in adults and infants.

Alterations of intestinal activity after birth can produce postnatal redistribution of transplacentally acquired drugs accumulated in the fetal intestine. Such redistribution can produce temporary elevations in blood levels during the neonatal period. The plasma concentrations in newborns whose mothers received metoprolol or other β-adrenergic blockers before delivery have been shown to rise 5- to 10-fold within 20 h after birth (Lindeberg et al., 1987).

Metabolism

Although some chemicals are eliminated from the body unchanged, most are converted to a wide variety of metabolites before they are excreted in urine, bile, or breath. The ability of newborns and infants to detoxify and excrete chemicals is critically dependent on the relative maturity of enzyme transformation systems. In general, most hepatic biotransfomation enzymes do not reach adult levels until after birth, but a few are more active prenatally than after birth (e.g., placental forms of glutathione transferase and γ-glutamyltransferase) (Dutton, 1982). An important aspect of metabolizing enzymes is their multiplicity. Families of isoenzymes carry out each type of biotransformation reaction. The expression of isoenzymes is both species- and strain-dependent and the development of isoenzymes follows different courses, producing different patterns of age-related changes in metabolizing capability (Gregus and Klaassen, 1998).

Both Phase I and Phase II biotransformation activities gradually develop during fetal life at varying rates. At birth, both systems are generally immature and require additional postnatal maturation. The major Phase I metabolic system comprises various forms of the hepatic microsomal P450 enzyme families, CYP1, CYP2, and CYP3. The P450s interact typically with substrates and molecular oxygen to form hydroxylated metabolites or intermediates. The most important Phase II metabolic system (glucuronide conjugation) is catalyzed by uridine diphosphate-glucuronosyltransferase (UDP-GT). Probably next in importance is glutathione conjugation. The development of these enzyme systems in both laboratory animals and humans is briefly discussed below to illustrate the great complexity and substrate-specific maturation of hepatic metabolism.

P450-catalyzed oxidation. Most lipid-soluble foreign chemicals, including drugs, pesticides, and environmental pollutants, are metabolized in animals by cytochrome P450 enzymes (Gillette and Stripp, 1975). These enzymes are found mainly in the smooth endoplasmic reticulum (SER) of the liver and intestinal cells. Species differences in the development of SER in hepatocytes during gestation may affect P450-related

activities in fetal liver. Compared to other species, P450 enzymes develop rapidly in humans, appearing during the first half of pregnancy and having at birth about one-third the rate of adults (Pelkonen et al., 1973; Cresteil, 1987). P450 enzymes are virtually absent in rat fetuses and have been detected during the third trimester in rabbit and guinea pig fetuses, but with activities of only about 1% of the parent, rising to adult levels by 3–8 weeks after birth. This species difference appears to parallel the development of the liver endoplasmic reticulum (ER). In laboratory animals, the rough ER appears by 7–9 days of gestation, whereas the SER does not appear until after birth. In humans, the SER is considerably developed by 3 months of gestation (Zamboni, 1965).

For some substrates, the adult activity of P450 enzymes is surpassed by that of neonates. For example, the infant at birth has about 30% of the adult capacity to metabolize phenytoin, a drug used to treat convulsions in infants. Within several weeks, however, phenytoin metabolism exceeds that of adults. Similarly, a rapid increase in phenobarbital metabolism shortly after birth follows an initially long serum half-life, with large interindividual variability during the first 5 days of life; adult metabolic rates are exceeded by 1 month of age (Neims et al., 1976).

Glucoronidation. Glucoronidation is quantitatively the most important conjugation reaction. Typically, compounds with phenolic (acetaminophen), alcoholic hydroxyl (chloramphenicol), or carboxyl groups (valproic acid) are conjugated by glucoronide. The enzymatic reaction is catalyzed by UDP-GT, using UDP-glucuronic acid as the cosubstrate. UDP-GT resides in microsomal membranes with its active site in the lumen of the endoplasmic reticulum (Clark and Burchell, 1994).

The prenatal and early postnatal development of hepatic UDP-GT activity takes different courses depending on both species and substrate. In the rat, at least three clusters of different UDP-GTs can be distinguished (Gregus and Klaassen, 1998). These clusters differ with respect to substrate specificity, inducibility, and maturation rate. The enzymes in the fetal cluster, whose activity reaches adult levels or even higher at birth, conjugate simple phenols (e.g., 4-nitrophenol, 1-naphthol, and 5-hydroxytryptamine). The UDP-GTs in the neonatal cluster exhibit low activity at birth, increasing gradually and reaching adult levels by 4 weeks of age. Somewhat larger molecules, like morphine, bilirubin, and chloramphenicol, use this pathway and similar patterns are seen in humans. For example, in human infants the half-life of morphine is 6–14 h compared to 2–3 h in adults (Kopecky and Koren, 1998) and bilirubin metabolism develops rapidly, reaching adult levels by 8–15 weeks (Rane et al., 1973). UDP-GTs in the perinatal cluster remain low throughout lactation and increase to adult levels only after weaning. Digitoxigenin monodigitoxoside and androsterone are conjugated by these enzymes. In fetal or neonatal human liver, UDP-GT activities for some of these compounds (e.g., 1-naphthol, 4-nitrophenol, 2-aminophenol, bilirubin, androsterone, and testosterone) are negligible or undetectable.

Glutathione conjugation. Glutathione (GSH) is the most abundant thionucleophile in cells. Compounds bearing an electrophilic atom are potential substrates and are often reactive as well as toxic; GSH is therefore significant in the activation and deactivation of electrophilic chemicals. The metabolic reactions are mediated by glutathione S-transferases and the resultant GSH conjugates are excreted in bile. GSH conjugation is one of the more important enzymatic antioxidant defenses against both xenobiotics and cell-generated peroxides.

Depending on the substrate and species, glutathione transferase activities follow different developmental patterns, reflecting differential rates of production of the different isoenzymes (Gregus and Klaassen, 1998). Maturation of GSH metabolic capacity is complex; the transferases are binary combinations of at least 12 protein subunits belonging to four multigene families. The resulting dimeric isoenzymes have overlapping substrate specificities and mature at different rates. Glutathione S-transferases appear in the liver of rats during midgestation. Their activity increases from a very low level to substantial levels at term, reaching adult levels at 2–4 weeks (Kashiwada et al., 1991). In contrast to laboratory animals, human fetal liver contains high glutathione transferase activity toward various halogen compounds, epoxides, and α, β-unsaturated compounds, approaching or even exceeding adult values. Diminished levels of GSH have been reported between 24 and 48 h after birth, both in term and in premature newborns, suggesting a common period of diminished antioxidant protection (Jain et al., 1995).

Excretion

Both renal and biliary excretion pathways have diminished capacity at birth and compounds eliminated by those routes may accumulate to higher levels in the neonate. Prior to birth, maternal and fetal blood are in close equilibration and for most substances that cross the placenta easily, fetal and maternal blood levels are similar. Birth creates a completely new situation for the offspring with respect to the elimination of substances. At birth, elimination of compounds across the placenta ceases and with the collapse of placental circulation, hepatic blood flow and oxygen supply dramatically decrease. Elimination of transplacentally acquired drugs slows considerably in the neonate compared with the fetus. The concentrations of drugs like ethanol, salicylate, and diazepam rise considerably in neonatal
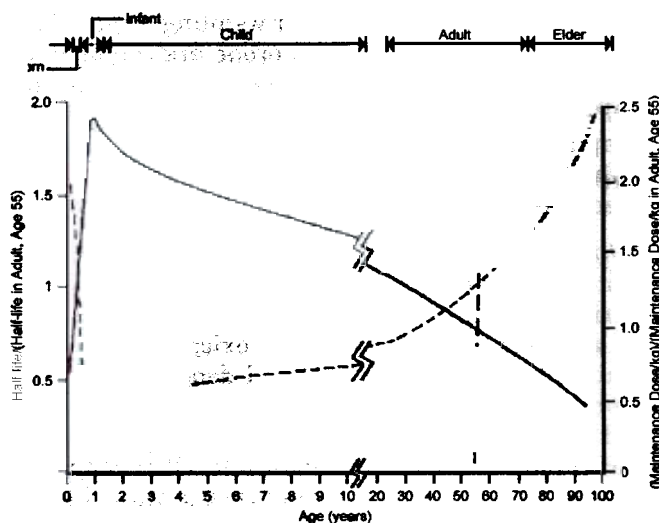
FIG. 2. Half-life, maintenance dose/kg or clearance dose/kg of a drug versus age, expressed as a fraction of the average half-life, maintenance dose/kg, or clearance/kg of a typical adult patient (55 years). The dose regimen assumes that the desired average plateau concentration of the drug remains the same throughout life. (Reprinted from Malcolm Rowland and Thomas N. Tozer, Clinical Pharmacokinetics: Concepts and Applications, Lea & Febiger, 1980)

blood as their elimination becomes dependent on the oxygen-deprived liver metabolism and reduced elimination capacity of the newborn (Hill et al., 1983; Levy and Garrettson, 1974; Cree et al., 1973). Disruption of the fetal–maternal blood flow also reduces the clearance of endogenous compounds produced by the fetus and eliminated by the liver.

Clearance, if normalized for body weight, is lower in the newborn and rapidly increases to reach a maximum at about 6 months, when it is almost twice that of the adult. The low rate of clearance around birth (3 ml/min) is one of the reasons why the half-lives of many chemicals are prolonged during this period. Figure 2 shows a plot of the half-life of a hypothetical drug with a clearance and half-life similar to creatinine. Creatinine distributes in total body water and is eliminated entirely by renal excretion through glomerular filtration. Because of immature kidney function, elimination is slow in the newborn, but by 1 year it has developed to the point that the half-life is one-half the adult value. Thereafter, weight-normalized clearance falls but still remains considerably above the adult rate during childhood.

The amount of a substance that is filtered by the kidney depends on renal blood and plasma flow. Renal excretion is dependent on GFR, tubular reabsorption, and tubular secretion. All of those processes are low at birth and increase rapidly thereafter. A decrease in any one of those processes in neonates can result in delayed clearance of a chemical from the body. For example, aminoglycoside antibiotics, such as kanamycin and gentamicin, are primarily eliminated without metabolism by glomerular filtration. The total body clearance of those

compounds in preterm and full-term infants is only about 5 and 20% of the adult values, respectively. Renal blood flow is low at birth because the kidney receives a lower proportion of the cardiac output and because of its high intrarenal vascular resistance; renal blood flow increases to adult levels by 5 month of age (Calango and Rubin, 1963). The GFR in premature newborns may be less that 5% of the adult value. The GFR for a term infant ranges from 2 to 4 ml/min, but increases within the first few days to 8–20 ml/min, attaining adult values by 3–5 months.

Glomerular function is more advanced at birth than tubular function. Maturation of tubular function is relatively slow, not reaching adult capacity until about 8 months. Compounds like the penicillins, which are eliminated primarily by tubular secretion, typically exhibit prolonged half-lives in newborns (Morselli et al., 1980). The deficient transport process and reduced GFR produce smaller medullary solute gradients, which result in a diminished capacity of the neonate to concentrate urine.

Studies have shown that, at birth, all aspects of the enterohepatic circulation, including bile synthesis, conjugation, transport, secretion, and reabsorption, are immature (Chuang and Haber, 1998). Because greater than 95% of the bile acids secreted from the adult liver are reabsorbed from the small intestine, elimination by this route can be compromised until maturation, which usually occurs after weaning in animals and by 1 year in the human. Many compounds administered to pregnant animals attain high concentrations in fetal liver (Waddel and Marlowe, 1976). Metals such as iron, zinc, and copper accumulate in the liver of human and animal fetuses and of neonates at levels considerably higher than in adult livers. Laboratory animals and humans of fetal and neonatal ages possess high levels of a hepatic cysteine-rich protein, metallothionein, which can bind cadmium and mercury, however (Clough et al., 1986).

CASE EXAMPLES

The following case examples that illustrate the many variations in the body's ability to handle different types of substances at different ages are described. In considering these examples it is apparent that many factors contribute to the differential susceptibility to toxicant exposures experienced by infants and children versus adults. Among the more important considerations are the age of the infant or child, the dose of the agent, and as the following examples emphasize, the nature of the chemical itself and its mode of metabolism. There is little doubt that if the doses are high and the infant is a neonate or still in the womb there is cause for concern over potential differential toxicity. But in other instances, particularly for low-level exposures and for infants 6 months or more of age, there is much less cause for concern.

Pharmaceutical Agents

Acetaminophen (Tylenol). When Tylenol began to be widely used in the 1960s, coincident with the increasing availability of self-medication, there was considerable concern over the possible vulnerability of infants to the drug. Numerous cases of acute liver toxicity in adults had been reported in the United Kingdom due to acetaminophen overdose, resulting in over 150 deaths annually (Prescott, 1983). In the 1970s, reports of toxicity and deaths from adult acetaminophen poisoning began appearing the U.S. literature (Rumack and Matthew, 1975). Acetaminophen was particularly attractive as an antipyretic for children because it could be formulated into a flavored liquid and avoided the potential side effects of aspirin. Several papers warned that the increased availability of the drug would increase the likelihood of accidental overdose in children and that increased morbidity and mortality were likely. However, despite careful observation of overdosed children, no deaths and very little toxicity occurred. Significantly, children exhibited less toxicity than adults, even when plasma concentrations of the drug were in the range that produced significant adult toxicity (Kauffman, 1992b).

Acetominophen undergoes metabolism by two major parallel pathways: conjugation by sulfate or by glucuronic acid. In addition, a small fraction of the drug is metabolized via a cytochrome-P450-mediated pathway to a highly toxic intermediate that reacts with GSH under normal conditions to form a nontoxic, stable conjugate. That conjugate is excreted as acetaminophen cysteine/mercapturate. However, when large, acute overdoses are ingested, the sulfation and glucuronidation pathways become saturated and a larger portion of the dose is metabolized via the P450 pathway, but insufficient GSH is available to detoxify the intermediate (Dahlin et al., 1984; Hinson et al., 1990). In children, there is a greater capacity both for GSH conjugation and for sulfation, resulting in a lower level of metabolism via the toxic pathway. Animal studies show that the rate of GSH synthesis in young animals is four times that of mature animals (Lauterburg et al., 1980). The less severe and lower rate of acetaminophen toxicity in children is believed to be due to a combination of higher GSH turnover and increased capacity to metabolize the drug via nontoxic conjugation pathways. Because P450 activity is low in neonates, decreased susceptibility to agents that are activated via P450 enzymes is anticipated. This is true for acetaminophen, bromobenzene, and carbon tetrachloride, none of which produces liver injury in neonatal animals at dosages that are hepatotoxic to adults (Gregus and Klaassen, 1998).

Chloramphenicol. During the 1950s, when chloramphenicol was first widely used for the treatment of certain refractory infections, it sometimes produced a pal-

lid cyanosis, gray baby syndrome, which progressed to circulatory collapse and death in some newborns (Sutherland, 1959). This problem occurred as a consequence of administering excessive doses, resulting from a failure to appreciate the diminished hepatic glucuronide conjugation capacity in newborns. Weiss et al. (1960) reported blood half-lives for chloramphenicol of 26, 10, and 4 h for newborns, 10- to 16-day-old infants, and 4- to 5-year-old children, respectively. Mulhall (1986) showed that the plasma clearance of chloramphenicol increased sixfold from age 30 to 140 days. Thus there is a substantial increase in chloramphenicol metabolism and excretion in the first days and weeks of life, with the daily dosage tolerated increasing from 25 mg/kg (newborn) to 300 mg/kg (older child) (Evans and Kleiman, 1986).

Drugs of Abuse

Ethyl alcohol. Use of alcohol during pregnancy is the leading cause of developmental impairment and deficits in children. The resulting fetal alcohol syndrome (FAS) includes a pattern of pre- and postnatal growth retardation, neurologic and cognitive impairment, and characteristic facial dysmorphology in the offspring of women who drink heavily before and during pregnancy. FAS occurs in approximately 1 to 2 infants per 1000 live births (Sokal, 1980). Full expression of FAS generally occurs with long-term consumption of at least 2 g of alcohol/kg bw/day (6–8 drinks per day), but the risk of intrauterine growth retardation has been reported to be increased by as few as 1–2 drinks per day (Little, 1977). A prospective study of 359 neonates that assessed the critical window of susceptibility to alcohol exposure demonstrated that cranial abnormalities are related to first-trimester ethanol exposure (Streissguth et al., 1989). Ethanol exposure during the third trimester increased the risk of postpartum ethanol withdrawal (Landesman-Dwyer et al., 1978).

The primary enzymes that oxidize alcohols, including ethanol and retinol, are the NAD-dependent alcohol dehydrogenases, which are zinc-containing proteins residing in the cytosol. Alcohol dehydrogenase activity increases with age in both laboratory animals and humans. Hepatic alcohol dehydrogenase levels in midterm human fetuses are 30% of the adult levels and elimination of ethanol is considerably slower than in their mothers (Smith et al., 1982). Neonatal toxicity and death have been associated with acute transplacental alcohol intoxication, in which decreased alcohol elimination may have been a contributing factor (Jung et al., 1980).

The level of blood alcohol achieved depends on the type of beverage (e.g., beer slows absorption), gastric contents, and gastrointestinal motility. Absorption is rapid, with 80–90% absorption of ethanol occurring within 30–60 min. Ethanol easily crosses the placenta,

cell membranes, and the blood–brain barrier. The volume of distribution is 0.6 liters in adults and 0.7 liters in children, corresponding to the volume of total body water.

The mechanism of adult alcohol intoxication is still not established. Early theories considered that alcohol dissolved in lipid membranes, perturbing the function of channels and embedded proteins. More recently, attention has focused on the receptor-mediated effects of ethanol on excitatory (glutamate) and inhibitory (GABA) amino-acid-activated ion channels. The mechanism of the fetal effects is still more complex and uncertain. There may be several fundamental mechanisms of ethanol teratogenesis. Free radical damage, altered prostaglandin metabolism, and interference with retinoid homeostasis have all been suggested as possible mechanisms (Stumpf, 1998). Duester (1994) suggested that ethanol acts as a competitive inhibitor of alcohol dehydrogenase (ADH), compromising retinol conversion to retinoic acid, which uses the same enzyme. Retinols play an important role in the development of the nervous system, modulating homeotic genes that convey positional information in the neuroaxis. Several studies indicate that retinoids are the long sought-after "morphogens" that convey positional information, thereby directing embryonic development (Maden, 1994). The neural anomalies produced in humans by large doses of ethanol and retinoids are similar and those agents share a vulnerable period in organogenesis. The competition of primary alcohols with retinol may account for their teratogenicity and for the lack of such an effect by secondary alcohols, which do not require ADH for their metabolism to ketones.

Environmental Agents

Lead. Lead is a cumulative toxicant. The daily intake of lead from food, water, and air accumulates primarily in the skeleton (~200 mg) in equilibrium with blood (~1 mg) and other tissues (Rabinowitz et al., 1976). Infants can be exposed to maternal lead via the placenta during gestation. Children are more sensitive to lead toxicity than adults. For example, the sensitivity of children to impaired CNS function from lead is approximately four-fold greater than that of adults (Davis and Grant, 1992). The differential sensitivity is due in large part to the greater absorption of lead from the GI tract. The EPA has shown that the likelihood of childhood lead exposure far exceeds that of adults (Davis and Grant, 1992). As a result, children are not only inherently more sensitive to lead, but also more likely to be exposed to higher doses.

The realization that early neonatal age is a critical period for metal accumulation and toxicity, including for lead, began in the early 1970s (Kostial et al., 1971; Forbes and Reina 1972; Chisolm, 1974; Momcilovic and Kostial, 1974). Experiments using suckling rats demonstrated the greater absorption and retention of lead by younger animals (Kostial et al., 1978). Lead has also long been known to affect several critical enzymatic reactions involved in heme synthesis; δ-aminolevulinic acid dehydratase is uniquely sensitive to lead level, with no apparent threshold for its inhibition (Roels et al., 1976; Roels and Lauwerys, 1987).

Chlorpyrifos. Chlorpyrifos (diethyl 3,5,6-trichlor-2-pyridyl phosphorothionate, Dursban) is an organophosphorus insecticide that, until recently, was widely used in agriculture, horticulture, termite control, and insect pest control (Racke, 1993; Richardson, 1995). Recent changes in its registration status have eliminated most of its nonagricultural uses. Chlorpyrifos is metabolically activated to chlorpyriphos oxon and to inactive TCP (3,5,6-trichloro-2-pyridinol). Chlorpyrifos oxon selectively and strongly inhibits acetylcholinesterase (AChE) in chlolinergic synapses. The resulting accumulation of acetylcholine and consequent chlolinergic hyperexcitation gives rise to the well-known signs and symptoms of acute organophosphorus poisoning. Chlorpyrifos oxon is hydrolyzed to TCP by A-esterases (Sultatos and Murphy, 1983a,b; Chambers and Chambers, 1989).

A number of studies have demonstrated that younger animals are more susceptible to acute, high-dose chlorpyrifos toxicity than older animals. However, more recent data have revealed that age-related differences arise from the limited capacity of younger animals to detoxify chlorpyrifos at very high doses. These apparent age-related differences are not observed at lower levels of exposure that are more consistent with environmentally relevant doses.

Mortensen (1996), using a peanut oil vehicle, obtained subcutaneous maximum tolerated doses for 7-day-old and adult Sprague–Dawley rats of 45 and 279 mg/kg, respectively, a six-fold difference. Whitney et al. (1995), using a dimethylsulfoxide vehicle and Zivic–Miller rats, obtained subcutaneous maximum tolerated doses (MTD) of 2 and 11 mg/kg in 1- and 8-day-old rats, respectively, a five-fold difference. Moser and co-workers (1996), using a corn oil vehicle and Long–Evans rats, found 17-day-old rats to be five times more sensitive than adults following oral exposure. Also by the oral route, Moser and Padilla (1998) found MTDs of 15, 20, 50, and 100 mg/kg for 10-day, 17-day, 27-day, and 70-day (adult) Long–Evans rats, respectively, which illustrated that the magnitude of the difference is greatest for the younger animals (Moser and Padilla, 1998). The four- to seven-fold greater sensitivity of the younger animals to chlorpyrifos appears to arise from the limited capacity of the biotransformation system to detoxify the oxon as well as from a lower capacity for synaptic compensation in the younger animals (Mattson et al., 2000).

The aggregate exposure to chlorpyrifos (dietary, indoor home, and outdoor) of young children aged

TABLE 2

Comparison of Cholinesterase Inhibition in Various Organs as a Function of Blood Concentrations (ng/g) of Chlorpyrifos (CPF) and 3,5,6-Trichloro-2-pyridinol (TCP) at All Times in Dams and Their Corresponding Fetuses and Pups

	RBC		Plasma		Heart		Fore brain		Hind brain	
	Dams	Pups	Dams	Pups	Dams	Pups	Dams	Pups	Dams	Pups
Blood concentration of CPF ng/g										
0	100	100	100	100	100	100	100	100	100	100
		106		96		94		91		100
	18		39		51		92		92	
12		15		40		35		63		
	1		6		11		11		20	
15	3		8		17		16		28	
46		8		15		18		40		46
109	5		12		17		13		24	
Blood concentration of TCP ng/g										
0	100	100	100	100	100	100	100	100	100	100
		97		96		100		102		
10	23		66		91		91		97	
25		111		98		101		98		104
45		86		91		94		97		96
49		57		82		84		103		88
71			49		35		23		43	
97		102		104		112		104		107
111	61		48		90		102		94	
114	74		67		100		98		101	
136		101		94		97		94		101
143	89		68		96		102		101	
322	18		39		51		92		92	
350		106		96		94		91		100
395	13		23		58		93		93	
537	16		31		69		90		88	
638		15		40		35		63		67
1450	2		8		17		16		28	
1782		8		15		18		40		46
1974	5		12		17		13		24	
2718	1		6		11		11		20	

Note. Cholinesterase values are as percentage of control (Source: Mattsson *et al.*, 2000).

0–6 years has been estimated to be between 0.24 and 1.2 μg/kg/day (Shurdut *et al.*, 1998). However, the MTDs from the laboratory studies described above are thousands of times higher than children's aggregate exposure. This discrepency has prompted investigation of age-related sensitivity to lower doses of chlorpyrifos. Pope and Liu (1997) showed that the differential sensitivity of young rats to high-dose exposures of chlorpyrifos did not appear to hold true for low-dose exposures. Mattsson *et al.* (2000) measured AChE inhibition in rat dams and pups after oral exposure to 0.3, 1.0, and 5.0 mg/kg/day from gestation day 6 to postnatal day 10. These authors found that pups were either less or similarly sensitive to AChE inhibition than dams in both blood and peripheral tissues when dose was measured as blood concentration of chlorpyrifos or one of its principal metabolites (see Table 2 and Fig. 3). Based on either similar or less AChE inhibition in observed fetuses and on further estimates of chlorpyrifos intake during nursing, neither fetuses nor neonates demonstrated greater
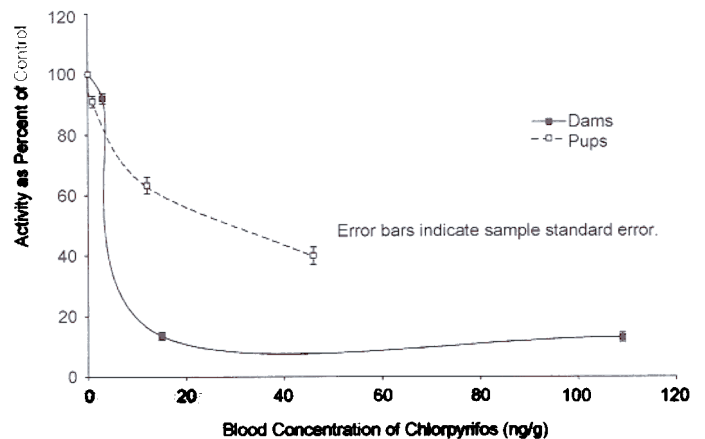
FIG. 3. Comparison of *fore brain* cholinesterase activity as a function of chlorpyrifos (CPF) blood concentration in dams and their corresponding fetuses or pups at all time points (based on Mattsson *et al.*, 2000).

sensitivity to AChE inhibition than their dams. Zheng et al. (2000) confirmed that the differential suscepti- bility to AChE inhibition between neonates and adults disappeared as the administered dose decreased to less than 1.0 mg/kg. Furthermore, it appears that the lesser fetal ability to detoxify the chlorpyrifos oxon is more than compensated for by the greater fetal ability to syn- thesize new AChE enzymes at low dose levels (Mattson et al., 2000). Thus, for environmentally relevant doses, the younger animals do not appear to be any more sen- sitive than the adults. These results are encouraging, but it has been suggested that prolonged, subtoxic doses of chlorpyrifos may exert direct actions on processes as- sociated with neural cell differentiation and replication that do not depend on brain cholinesterase (Johnson et al., 1998; Whitney et al., 1995).

Dietary Agents

Caffeine. Caffeine is one example of several sub- stances that are eliminated by hepatic transforma- tion whose elimination and transport are prolonged in neonates. Caffeine is commonly ingested by pregnant women in the form of chocolates, coffee, and soft drinks. Caffeine has been reported to be teratogenic in labora- tory animals at high doses; epidemiologic studies are conflicting (Christian and Brent, 2001). Both premature and full-term infants eliminate caffeine more slowly than adults (Aldridge et al., 1979). The plasma half-life in infants is about 4 days; in adults it is about 4 h.

The elimination of caffeine occurs through demethy- lation, which depends on the perinatally deficient en- zyme CYP1A2. The major metabolites of caffeine in adults are dimethylxanthines and mono- and dimethy- luric acids. Complete maturation of the metabolic path- way occurs gradually within the first 5–6 months of life (Aranda et al., 1981; Hakkola et al., 1994). The imma- turity of this metabolic pathway is reflected both in a longer half-life and in a larger fraction being excreted unchanged. In neonates as much as 90% is excreted in the urine as caffeine whereas in adults the fraction of unchanged caffeine is less than 1–2%. The magni- tude of this maturation deficit is larger than for other xenobiotics and is caused by several factors. First, caf- feine is metabolized exclusively by the hepatic mixed- function oxidase system and is eliminated according to an enzyme-limited clearance pathway. Second, there is some metabolic recycling. Finally, in addition to a de- creased rate of elimination, a larger volume of distribu- tion increases its half-life (Gregus and Klaassen, 1998).

Uneven development of biotransformation pathways in early life can result in the use of alternative path- ways that are unique in infants. The most striking example of this is theophylline. In adults and older children theophylline is largely N-demethylated to 3- methylxanthine by a P450-catalyzed reaction. This pathway is deficient in the newborn, although methyla-

tion is well developed. As a result, theophylline in early postnatal age is methylated to form caffeine. Both caf- feine and theophylline are slowly converted to mono- and dimethyluric acids.

Endogenous Agents

Bilirubin. Bilirubin is the end product of heme catabolism from circulating red blood cells and from other heme-containing proteins and enzymes. The degradation of heme to bilirubin also releases carbon monoxide. Compared with the adult or older child, new- born infants exhibit greater rates of bilirubin produc- tion and lower rates of elimination, leading to neonatal jaundice.

In the adult, the hepatic uptake, conjugation, and ex- cretion of both conjugated and unconjugated bilirubin are efficient and plasma bilirubin remains low. New albumin-bound bilirubin leaving the reticuloendothe- lial system is delivered to membrane receptors in the hepatic sinusoids where it dissociates from albumin. Within the hepatocyte bilirubin binds to a receptor protein generically known as ligandin (glutathione S- transferase A1) and is carried to the SER where it is conjugated and then excreted in the bile. The hepatic conjugating system is dormant until after birth and liver circulation is impaired for several hours. Conse- quently, nearly all newborns have some elevation of serum bilirubin and many have levels that would be ab- normal at any other time of life. Unconjugated bilirubin is a well-established central nervous system toxicant, although the exact mechanism is unknown (Cashore, 1998). The most likely mechanism involves the entry of unbound bilirubin into the central nervous system followed by bilirubin-induced disruption of several neu- ronal functions.

Physiologic jaundice in newborns may overlap with early-onset, breast milk jaundice. The consumption of human milk is now believed to be related to neonatal hy- perbilirubinemia (Schneider, 1986; Bracci et al., 1989). In a review of 12 studies involving 8000 infants in the first week of life, moderate jaundice was found in 12.9% of breast-fed infants and 4% of formula-fed infants. Se- vere jaundice was found in 2% of the breast-fed infants and in 0.3% of the formula-fed infants (Saigal, 1992). The etiology of breast milk jaundice is not clear; various hypotheses include inhibition of glucuronyl transferase by the free fatty acids or the pregnanediol in breast milk, promotion of bilirubin intestinal absorption by breast milk, and differences in the intestinal flora of breast-fed and formula-fed infants (Gourley, 1998).

CONCLUSIONS

Children, particularly neonates, can be biologically more sensitive to the same toxicant exposure on a body weight basis than adults. There is no doubt that

neonatal and infant humans have been harmed by exposures that do not harm adults who are similarly exposed. When children have been differentially harmed, most recent cases involve improper use or abuse of pharmacologically active substances by their parents. These include primarily the excessive consumption of alcohol or other pharmaceutically active agents during pregnancy. High doses of pharmacologically active substances and exposure during pregnancy are the common characteristics of these exposures. We are not aware of reported cases of differential harm to infants or children from low levels of regulated chemicals, like pesticides or food additives. Some environmental contaminants like lead may accumulate in the body and children's exposures have been and remain a concern, particularly in some older cities with lead-painted structures. Many persistent organic compounds like PCBs have been banned or rigorously regulated, like dioxins; their environmental levels have been reduced dramatically and continue to decline.

Virtually everything we know about differences in the susceptibilities of infants, children, and adults comes from relatively high-dose studies with drugs, from intentional or accidental exposures (to high doses), from substances that accumulate to high doses after repeated exposures, and from improper use or abuse. There is little in the literature suggesting that low doses of chemicals that are modestly well excreted are more hazardous in infants and children than in adults (e.g., for an exception, however, see nitrate). Data from one of the anti-cholinesterase insecticides (chlorpyrifos)—one of the few chemicals for which low-dose data exist—indicate that infants are more sensitive than adults to high, acute, or repeated doses and less or similarly sensitive to low, repeated doses. Low environmental exposures to chemicals are less likely to overwhelm developing detoxification and elimination mechanisms so, where they occur, age-related differences at low doses may be quantitatively less pronounced than at high doses.

Current understanding of the rates of maturation of metabolic capability and evidence from case examples indicate that human infants up to approximately 6 months of age are typically—but not always—more sensitive to chemical toxicity than adults. For most chemicals, the immaturity of infant biotransformation, elimination, and other physiologic systems usually produces higher blood levels for longer periods. There is metabolic capacity for most tested substances in the newborn, although it is quite low and immature for some chemicals. For some chemicals, unique metabolic pathways not available in the adult human can also be utilized by the newborn. The newborn's metabolic capacity rapidly matures and, by about 6 months of age, children are usually not more sensitive to chemical toxicity than adults. By then, most metabolic systems are reasonably mature, becoming almost completely capable by 1 year of age. Children over 6 months of age can be more sensitive to chemical toxicity than adults (e.g., caffeine), but they usually are not; in many cases they are less sensitive.

Whether children are at greater risk from chemical exposures is another question. Risk depends on both inherent sensitivity and exposure conditions. If chemical exposure levels remain below those capable of overwhelming a child's metabolic detoxification systems and producing toxicity, children will be at no greater risk than are adults. Children under 6 months of age have little exposure to solid food, do not yet crawl around, and are not yet mouthing everything around them, so their exposures to environmental pesticide residues, for example, may remain less than those of older children. Children of all ages are still developing, of course, so even if they are exposed to chemicals at levels below those of adults, they may be at greater risk than adults. However, as long as those exposure levels are still below those required to produce toxicity, children will not be at greater risk.

The goal of regulatory safety assessment is to ensure that children and adults are protected from chemical risks. Such safety assessments are based on information about potential hazard, the relationship between dose and response, and anticipated or, in some cases, upper-bound estimates of exposures. For regulated chemicals like food additives or pesticides, exposure is limited by regulatory tolerances. Such tolerances generally are based on animal tests and limit allowable exposures to below those doses shown to have no effect in test animals. If tolerances are determined accurately and, in particular, by tests in which the animals are exposed prenatally and throughout their complete lifetimes, humans of whatever age, exposed at or below the tolerances, will not be at significant risk. Safety assessments of pharmaceuticals, nutrients, pesticides, and industrial chemicals may differ, however, depending on the potential for children's exposures.[2]

In the second part of this analysis we examine the adequacy of the testing protocols and uncertainty factors used by regulatory agencies to limit chemical exposures. As our knowledge about child-specific exposure levels and behaviors and about potential developmental hazards continues to improve, our confidence in the ability of regulation to provide appropriate protections will also improve.

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[2] See Dourson et al. (2002) for a discussion of the use of tiered toxicity testing strategies to set testing priorities based on both toxicity concerns and exposure potential.

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