The Role of Environmental Hazards in Premature Birth: Workshop Summary

Donald R. Mattison, Samuel Wilson, Christine Coussens, and Dalia Gilbert, Editors, Roundtable on Environmental Health Sciences, Research, and Medicine

ISBN: 0-309-52726-0, 148 pages, 6 x 9, (2003)

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Roundtable on Environmental Health Sciences, Research, and Medicine

Board on Health Sciences Policy

INSTITUTE OF MEDICINE
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington, D.C.
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THE NATIONAL ACADEMIES PRESS • 500 Fifth Street, N.W. • Washington, DC 20001

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Support for this project was provided by the National Institute of Environmental Health Sciences, National Institute of Health (Contract No. 282-99-0045, TO#5); National Center for Environmental Health and Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention (Contract No. 200-2000-00629, TO#7); National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention (Contract No. 0000166930); National Health and Environment Effects Research Laboratory and National Center for Environmental Research, Environmental Protection Agency (Contract No. 282-99-0045, TO#5); American Chemistry Council (unnumbered grant); and Exxon-Mobil Corporation (unnumbered grant). The views presented in this book are those of the individual presenters and are not necessarily those of the funding agencies or the Institute of Medicine.

Library of Congress Cataloging-in-Publication Data

The role of environmental hazards in premature birth / Donald R. Mattison ... [et al.], editors.
p. ; cm.
"Proceedings of a workshop that was sponsored by the Roundtable on Environmental Health Sciences, Research, and Medicine"—T.p. verso. Includes bibliographical references. Support for this project was provided by the National Institute of Environmental Health Sciences, National Institute of Health (contract no. 282-99-0045, TO#5) ... [et al.]
ISBN 0-309-09065-2 (pbk.); ISBN 0-309-52726-0 (PDF)
1. Infants (Premature)—Congresses. 2. Infants (Premature)—Environmental aspects—Congresses. 3. Environmental health—Congresses.
[DNLM: 1. Labor, Premature—etiology—Congresses. 2. Environmental Exposure—adverse effects—Congresses. 3. Hazardous Substances—adverse effects—Congresses. WQ 330 R745 2003]
I. Mattison, Donald R. II. Institute of Medicine (U.S.). Roundtable on Environmental Health Sciences, Research, and Medicine. III. National Institute of Environmental Health Sciences.
RJ250.R64 2003
618.92'011—dc22
2003018950

This summary is based on the proceedings of a workshop that was sponsored by the Roundtable on Environmental Health Sciences, Research, and Medicine. It is prepared in the form of a workshop summary by and in the names of the editors, with the assistance of staff and consultants, as an individually authored document.

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Printed in the United States of America.

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Willing is not enough; we must do."
—Goethe

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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the final draft of the

report before its release. The review of this report was overseen by **Melvin Worth**, Scholar-in-Residence, Institute of Medicine, who was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Preface

This workshop is the third of a series of workshops sponsored by the Roundtable on Environmental Health Sciences, Research, and Medicine since the Roundtable began meeting in 1998. When choosing workshops and activities, the Roundtable looks for areas of mutual concerns and also areas that need further research to develop a strong environmental science background. The Roundtable delegated the planning of this workshop to a planning group. The group was headed by Donald R. Mattison and Samuel Wilson, who were involved in the editing of this summary.

Like many organizations and health agencies, the Roundtable is interested in children and other special populations that may be particularly vulnerable to environmental exposures. Through basic research, the members of the research community and policy makers have begun to address the needs of children and environmental exposures. There is a growing understanding, based on their physiology and metabolism, that the effects of food, drugs, and environmental exposures are vastly different between children and adults. For example, the rate at which a child absorbs lead, a known neurotoxicant, from the gastrointestinal tract is higher than that of adults. These differences in environmental exposure can have a dramatic impact on the child's health and well-being throughout his/or her life.

In response to the need for healthy starts, the U.S. government has created several agencies that are charged with improving the health of mothers and children. In May 1997, the U.S. Environmental Protection Agency (EPA) established the Office of Children's Health Protection (OCHP). Its mission is to make protection of children's health a fundamental goal of public health and environ-

mental protection in the United States. The Maternal and Child Health Bureau (MCHB) of the Department of Health and Human Services (DHHS) provides leadership, partnership, and resources for advancing the health of all of our nation's mothers, infants, children, and adolescents. The National Center on Birth Defects and Developmental Disabilities (NCBDDD) at the Centers for Disease Control and Prevention (CDC) seeks to promote fetal, infant, and child development and to prevent birth defects. The National Institute of Child Health and Human Development (NICHD) at the National Institutes of Health (NIH) seeks to ensure that every individual is born healthy and wanted, that women suffer no adverse consequences from the reproductive process, and that all children have the opportunity to fulfill their potential for a healthy and productive life unhampered by disease or disability. These agencies perform an excellent service in focusing on children's and maternal health issues.

As many of the speakers noted during the workshop, the first environment that people are exposed to is the uterus. It is there that the fetus undergoes a profound development from a single cell to an infant in nine months, and there are many opportunities for problems to occur because of gene–environment interactions. The health community is still at the point at which additional research is needed to understand these interactions and their effects on health. During this workshop, numerous individuals highlighted the growing problem of preterm birth and the idea that it has many potential causes. Although some preterm births are medically indicated,* the vast majority are spontaneous—occurring for unknown reasons. The current estimates suggest that preterm birth occurs in approximately one in eight births, accounting for about 75 percent of neonatal deaths and contributing to approximately 50 percent of the long-term neurological damage in children in this country. Babies who survive the odds of preterm birth often spend months, or even years, overcoming illnesses and fighting for survival.

To protect children more effectively from the consequences of being born preterm, the health community must examine the role of social and behavioral factors—such as stress, anxiety, depression, drugs use, alcohol use, and tobacco smoking—in placing a woman at risk for delivering a premature baby. The health community also needs to know what types of environmental exposures adversely affect pregnant women and their unborn children and which women are at particular risk. The challenge is difficult, but it can be met. Our past successes can guide us. In the 1970s when lead exposure dominated the headlines, as a member of Congress I held hearings to address the problem. When presented with solid scientific evidence, Congress responded, and changes in the law resulted in decreased blood lead levels.

* Medically indicated refers to the intervention of a clinician because the fetus and/or mother are in danger of dying.

This workshop provided the Roundtable members, speakers, and participants the opportunity to take a critical look at the issue of preterm birth as an environmental health problem. There are still a number of gaps in the knowledge of basic mechanisms of labor and delivery, as well as the interactions that might contribute to preterm birth. During the formal and informal discussions, many individuals commented on the need to have places where researchers across the scientific disciplines can gather to find areas of commonality and opportunities for future collaborations.

I was struck by the end of the workshop that the researchers had made significant progress in this area and there is solid science that is beginning to unravel the mysteries of preterm birth. It was clear that researchers and policy makers has to continue to support policy that is based on solid science grounding. Finally, the public need to remember that children do not have the capacity to make informed decisions on their own. They rely on us—researchers, scientists, policy makers, and community groups—to be their voice and to protect them.

Paul G. Rogers, J.D.
Chair

Contents

Summary*

The Roundtable on Environmental Health Sciences, Research, and Medicine is comprised of key stakeholders in environmental health who meet on a regular basis to discuss areas of mutual concern in a neutral environment. The purpose is to promote discussion, but not to come to consensus. Sometimes, the Roundtable convenes workshops to explore issues in greater depth and facilitate discussion. This is a summary of a workshop convened by the Roundtable on October 2–3, 2001, to look at the issues surrounding the role of the environment in premature birth. The summary has been prepared by the workshop rapporteur as a factual summary of what occurred at the workshop and should not be construed as consensus by the Roundtable or the Institute of Medicine.

PRETERM BIRTH

Each year in the United States, more than 440,000 babies are born too soon. These preterm births, defined as those occurring before the thirty-seventh week of pregnancy, represent nearly 12 percent of all births. Premature infants can be dangerously small—weighing less than 2,500 grams—and are at greater risk of death and lifelong disability than those born at full term. About 75 percent of infant deaths in the first month of life occur in premature infants, who are much more likely than full-term infants to have breathing problems or to suffer life-

* This summary was prepared from the transcript of the meeting by Christine Coussens and Kathi Hanna as the rapporteurs.

long medical complications such as cerebral palsy, visual and hearing disabilities, and mental retardation.

Premature babies often spend months fighting for survival and struggling to overcome illness. The impact on the families is often long-lasting and results in emotional and financial hardships. The enormous medical costs associated with preterm birth, during infancy and often continuing throughout the child's life, represent about 35 percent of all medical expenditures for newborns and about 10 percent of all medical expenditures for children. Some estimates indicate that in the first year of life alone, a preterm birth costs about $15,000 more than a full-term birth.

Although vast improvements have been made in preventing deaths of premature infants, little success has been attained in understanding and preventing preterm birth, and the knowledge that has been gained about preterm labor has not translated into improved perinatal outcomes. Despite all efforts to reduce the condition, the rate of preterm birth has increased during the past 20 years. Since the early 1980s, the rate of preterm birth in the United States has increased by 17 percent, and the incidence of low birth weight (less than 2,500 grams) has risen 10 percent. For reasons that are not fully understood, these problems take a disproportionate toll on African Americans and recent immigrants from Latin America. Such demographic differences have led many to characterize preterm birth as a disease of poverty.

Attempts have been made to reduce the prevalence of the conditions leading to preterm birth, especially preeclampsia and fetal growth restriction. Interventions such as improved nutrition, maternal bed rest, low-dose aspirin, and calcium supplementation have been tried, but they have generally failed. Evidence suggests that although the prevalence of preeclampsia and fetal growth restriction appears to have remained unchanged, early delivery for fetal distress associated with these conditions has reduced stillbirths.

Clearly, preterm birth is not an acute event. Its roots may well begin before pregnancy, perhaps even in a woman's early life. The discussion of preterm birth is confused by a lack of firm agreement about, and understanding of, what constitutes "preterm." The length of the natural term of pregnancy exhibits tremendous variability, and the gestational period for an infant with low birth weight can be within a normal range. Further, the assignment of due dates for delivery is an inaccurate clinical practice. In fact, only 4 percent of all women deliver on their due date.

Normal pregnancy is a carefully programmed sequence of events from the perspective of uterine activity, in which one genetic function leads to another. In general, pregnancy can be divided into four stages. Most of the pregnancy is a stage of uterine quiescence, maintained by active mechanisms. This stage is followed by a stage of preparation or activation, when the contraction-activating proteins are turned on by a variety of mechanisms that prepare the uterus to become a contractile organ. The next stage, onset of labor, requires an integra-

tion of endocrine and mechanical signals to initiate and maintain uterine contractility. Parturition at the appropriate time is the final stage of a successful pregnancy.

In a normal pregnancy, fetal maturation is closely synchronized with uterine quiescence to prevent birth before the fetus is viable. Many factors have been identified that contribute to uterine quiescence and contractility, and many markers have been identified that provide clues as to how disruptions in the many pathways that lead to labor and delivery can cause them to go awry. Several approaches have been taken in animal and human studies to assess the roles of endocrine hormones, steroids, immune responses, and nitric oxide in labor and parturition. The mechanisms affecting uterine contractility and cervical ripening have also been examined. Basic discoveries from these investigations provide some clues as to how environmental perturbations may affect the timing of parturition and the duration of labor. However, much of what has been learned about labor and parturition has come from experiments with sheep and other animal models. Although these findings cannot be completely extrapolated to humans, they do provide important insights about what underlies normal and abnormal parturition in humans.

Epidemiological research on the association between preterm delivery and such factors as life-style behaviors (e.g., tobacco use, cocaine use, alcohol use, physical inactivity), nutrition (e.g., lack of iron or folate), infection (e.g., bacterial vaginosis), and psychological stress (e.g., anxiety, depression) has been under way for some time. This research suggests possible etiologic pathways to preterm birth, offers insights into methodological challenges, and identifies potential confounding factors. At present, strong predictors of preterm birth are limited to multiple gestation, prior preterm birth, and African-American ethnicity; weaker but modifiable influences include infection, tobacco use, low prepregnancy weight, and lower socioeconomic status.

Numerous studies in humans have suggested the strong predictive value of social and psychological stress in initiating preterm labor. Studies at the tissue, cellular, and molecular levels have provided some preliminary understanding of how social and environmental stressors can disrupt the normal pathways that lead to labor and parturition. However, some workshop participants noted that more information is needed on interspecies differences and on critical periods in, and mechanisms for, the initiation of labor.

Some workshop participants stressed the importance of improved interactions across disciplines—particularly among epidemiologists, reproductive biologists, and toxicologists—to elucidate the root causes of preterm birth (see summary box for a listing of research opportunities). Epidemiologists can identify associations of exposures and risks, but they are not likely to identify causal relationships. Reproductive biologists and toxicologists can provide biologically plausible mechanisms that may explain these epidemiological associations. In addition, studies in which reproductive biologists and toxicologists collaborate

BOX S.1
Major New Research Opportunities

During the workshop, participants listed a number of research opportunities for research in preterm birth. These included the following:

1. Improved animal models for the various etiologies of premature birth.
2. Molecular classification of preterm stages by genomics-based assays.
3. Molecular classification of exposures by improved genomic-based assays.
4. Further definition of biological pathways involved in term and preterm birth.
5. Improved surveillances and registries.

could lead to the identification of potential chemical hazards, and epidemiological studies could reveal the relevance of these other studies to humans.

Experience suggests that multidisciplinary collaborations are greatly needed to assess comprehensively the role of social and environmental factors, genetic factors, and gene–environment interactions in influencing preterm births. The gene–environment approach is likely to improve knowledge of the pathogenesis of preterm birth and to help develop a generic analytical approach to understanding the genetic contribution in populations with marked differences in social status and experience.

Conventional interventions to reduce spontaneous preterm birth include prenatal care, across-the-board nutritional supplementation, and social support or home visiting. More targeted, experimental interventions may include specific aspects of prenatal care, such as risk screening, nutrition counseling, caloric supplementation, protein supplementation, iron supplementation, labor-inhibiting agents, bed rest, hydration, home uterine activity monitoring, and drug, alcohol, and tobacco cessation programs. However, these types of interventions have rarely been shown to be beneficial in reducing the rate of preterm birth. In fact, there is little evidence that any of the numerous strategies currently used to reduce preterm birth are effective when applied to various populations.

The ability to predict individuals at higher risk of preterm birth without the ability to provide an effective treatment results in an increased use of ineffective interventions and higher costs, as well as a potential increase in iatrogenic complications. However, advances in molecular biology may lead to predictive "tests" to identify those at risk for preterm birth and to provide clues to potential therapies. Workshop participants generally agreed that the desired outcomes of interventions would be reduced mortality of the mother and reduced mortality or morbidity of the infant, reduced incidence of long-term disability in children, and reduced rates of severe neonatal morbidity.

Some workshop participants questioned whether the disappointing trend toward an increase in preterm birth rate reflects a failure to prevent preterm delivery, or whether it results from relying on the increased use of early ultrasound for estimating gestational age, early delivery for extreme fetal growth retardation and severe preeclampsia, multiple gestation, inclusion of newborns near the borderline of viability on registries, or changes in sociodemographic or behavioral determinants of preterm birth. These questions can be answered, many participants concluded, by conducting carefully designed interdisciplinary studies that account for the environmental influences and biological mechanisms that cause a pregnancy to end prematurely, placing the fetus, and sometimes the mother, at increased risk.

Charge to Participants and Workshop Scope*

Donald R. Mattison

Preterm birth is one of the more complex and challenging chronic diseases that have captured the attention of the life sciences and environmental health sciences. Worldwide, preterm births—those that occur before 37 weeks of gestation—constitute 5 to 12 percent of all births, accounting for more than 400,000 births each year. In the United States alone, more than 1,200 babies are born prematurely every day, and the rate of prematurity in this country is increasing.

Our interest in preterm birth springs from the fact that children who are born too early suffer adverse consequences. One potential consequence of prematurity is respiratory distress syndrome, which impairs the areas of the lung over which oxygen transport and diffusion take place. Another possible consequence is brain damage, often from intraventricular hemorrhage and periventricular cysts, which can lead to neurodevelopmental problems. In industrialized and nonindustrialized countries alike, preterm birth is linked to morbidity and mortality. Clearly, we need to investigate the causes of this condition.

In the past, prematurity was defined by the infant's birth weight—less than 2,500 grams. Recently, we have recognized that weight alone does not adequately characterize the risks faced by premature infants. Currently, characterizing the status of a child at birth involves both birth weight and gestational length. Infants of greatest concern are those with a gestational length of less than 32 weeks and a birth weight of less than 1,000 grams, because they suffer the most severe consequences.

During this workshop, we will place preterm birth in a framework similar to

* This chapter is an edited transcript of Dr. Donald Mattison's summations at the meeting.

that for diseases such as diabetes or hypertension, which are influenced by multiple genetic, social, personal, and environmental factors. Although the biological reasons for differences across ethnic background have not been identified, certain populations have a substantially greater risk for prematurity, and prematurity rates vary by region across the country. For example, substantial differences between African Americans and Caucasians have been found in preterm birth rate and infant mortality associated with prematurity (Healthy People 2010). These differences are difficult to understand biologically, suggesting that environmental factors play a role. We must consider carefully what these environmental factors might be, because understanding the factors that influence these variations will help us develop a better sense of the cause of prematurity and strategies for preventing it.

Among the five leading causes of infant mortality, two—low birth weight and respiratory distress syndrome—are associated with being born too early. The economic costs of preterm birth are substantial, both at the beginning of life and throughout its course. Data from the Agency on Healthcare Research and Quality reveal that two of the five most expensive hospital conditions in the United States in 1997 were associated with prematurity (AHRQ, 1996). Respiratory distress syndrome, the most expensive condition, had a mean hospital charge of $68,000 and a length of stay of more than 24 days. Low birth weight did not lag not far behind, with a $50,000 mean hospital charge and a length of stay of more than 21 days. These data are for children who are discharged alive. We have begun experimenting with community interventions to try to understand how we can influence these outcomes.

Preterm deliveries fall into three broad categories or pathways. The first category is medically indicated early deliveries—those necessitated by maternal or fetal factors. In such cases, it is believed that whatever the potential consequences of prematurity may be, early delivery is much safer for both the mother and the child. The other two categories—early deliveries due to spontaneous preterm ruptured membranes and those due to spontaneous preterm labor—may respond to intervention. As ones knowledge of etiology improves, all of these pathways may yield to prevention. Some participants have asked that we consider the possibility that early deliveries in all three categories are modifiable. Some have suggested that the factors influencing early delivery in each of these categories may be the same. Thus, we need to examine whether the strategies for preventing early delivery in one category may prevent conditions associated with another category. One charge for this workshop is to consider why we have created separate categories, which may be artificial, and how prevention strategies may affect all categories simultaneously.

An underlying truth regarding prematurity that relates to the issue of separating individual, environment, and genetic factors is the unfortunate observation that the best predictor of having a preterm birth is having experienced one previously (Mattison et al., 2001). The risk increases with each successive preterm

birth. For example, if a woman's first child is born at term, her risk for preterm delivery of a second child is about 4 percent. However, if her first baby is born prematurely, her risk for preterm delivery of a second child jumps to 17 percent. If her first and second children are born prematurely, her risk for preterm delivery of a third child rises to almost 30 percent. Looking deeply into this situation may provide a key to understanding the factors associated with prematurity.

Some factors influencing prematurity have already been identified. Most studies have focused on the individual and have set out to explore the characteristics of individuals that might confer risk for preterm delivery, some of which may be modifiable and some of which may not. Skin color and age are examples of characteristics that cannot be changed. The consequences of socioeconomic influences, which might persist across a life span, may respond to intervention at some level, but they may not be modifiable to any great extent within the individual.

Emerging data suggest that other factors play a role in prematurity. Some of these data point to environmental factors, and these findings bring us back to the traditional public health paradigm in which we must distinguish between social, biological, and environmental factors and try to understand how they interact in the condition of prematurity. Within this context, the specific goals of this workshop are to

- summarize the clinical and epidemiological aspects of prematurity;
- create an understanding that exposures to environmental chemicals can alter gestation length;
- summarize cellular, molecular, and genetic aspects of control of preterm delivery;
- recognize that current in vivo and in vitro toxicological testing models are inadequately designed to capture the data showing whether chemicals influence gestation length;
- understand that, because preterm delivery is a substantial public health concern, toxicological approaches have to be developed to improve our understanding of the impacts of the chemicals on gestational length; and
- recognize that a multidisciplinary approach is needed to better clarify the mechanism underlying gestational length

Our overall goal is to begin to summarize the current understanding of prematurity from the particular perspective that each of us brings to this topic and to stimulate cross-disciplinary interaction.

Problem Statement*

Jennifer Howse, Ph.D.

Not long ago, discourse on preterm birth was nearly always centered on the role of the mother. Increasingly, however, environmental hazards are being recognized as contributors to the devastating and costly problem of preterm birth. One purpose of this conference is to help establish a framework that will allow us to broaden our perspective and link our knowledge about environmental hazards to their potential effects on preterm birth. The work of the Honorable Paul Rogers, chair of the Roundtable on Environmental Health Sciences, Research, and Medicine, has been a major catalyst for this wider perspective.

The Institute of Medicine (IOM) is another positive force that is helping to redefine our thinking about preterm birth and its causes, and we are honored that the IOM has chosen to lend its weight to the examination of this issue. Several other important organizations are also represented at this workshop, including the National Institute of Environmental Health Sciences, the Environmental Protection Agency, and the Centers for Disease Control and Prevention, all critical partners in research efforts to identify potential environmental and social contributors to preterm birth.

The interest of the March of Dimes in preterm birth is long-standing. Our efforts began in the early 1960s after the battle against polio had largely been won. Prevention of birth defects and infant mortality became the clinical components of our mission at that time, and we are proud to have been leaders in creating a blueprint for the establishment of neonatal intensive care units in the

* This chapter is an edited transcript of Dr. Jennifer Howse's summations at the meeting.

early 1970s. We also made contributions in research, particularly in respiratory therapy, which have helped extend the survival of infants in neonatal intensive care.

We can all take great pride and consolation in the sound record that we have achieved in this country since the early 1960s, and even earlier, in reducing infant mortality. Infant mortality rates have declined substantially since that time, although they have leveled off somewhat in recent years and 2010 targets are not yet in sight. However, juxtaposed against the declining infant mortality rate is the ever-increasing rate of preterm births, which rose from under 9 percent in 1980 to 11.6 percent in 2000, and which is considerably higher in the United States than in other industrialized countries. This increase is driven by a complex of factors that we are struggling to understand.

A possible approach to lowering preterm birth rates is to examine how they relate to race and ethnicity. An African-American baby's risk for being born prematurely is nearly double that for a Caucasian baby. The risk is also higher for babies born to Native American and Hispanic women. For some time, the IOM has been at the forefront of noting such disparities and has been exploring possible links between preterm birth and environmental hazards.

The IOM's 1999 report Toward Environmental Justice: Research, Education, and Health Policy Needs concluded that certain communities do tend to have higher levels of exposure to environmental toxicants. It also concluded that such exposure is compounded by various socioeconomic factors that often make these communities less able to deal with the problem, such as lower levels of education, higher levels of stress, and inadequate access to health care.

At the March of Dimes, we have believed for some time that progress toward understanding the biological and social aspects of labor and delivery needs to be accelerated. We have also noted that an epidemiological approach to understanding preterm labor has not yet received sufficient attention. During the past 10 years, the March of Dimes has invested resources in these issues. The investment was modest at first, but recently we have devoted many millions of research dollars to studying preterm birth.

In 1998, we initiated a focused perinatal epidemiological research portfolio called the Perinatal Epidemiological Research Initiative (PERI), an ongoing portfolio funded at just under $4 million. Through this portfolio, we seek to examine how the influence of external factors can be understood in terms of their biological plausibility. We did not expect the overwhelming response that we received from our request for proposals. More than 100 scientists from all over the world responded. We asked applicants to explain how they would conduct further exploration into the medical, psychological, and social factors that may combine with maternal and fetal biochemistry to lead to preterm birth. At the end of the review process, a panel of experts selected six promising epidemiological research projects for funding, including projects that focused on how elements such as infections, genetic predispositions, low socioeconomic status, stress, and

other factors can initiate pathways to preterm delivery. We have been very pleased with the PERI studies so far.

Preterm birth is a complicated public health problem for which answers will not be found in any one place. We have not yet definitively shown clear cause-and-effect relationships between environmental factors and preterm birth, and we do not yet fully understand what constitutes the fetal environment. Nevertheless, we are encouraged by the large amount of promising research currently under way, some of which is funded by agencies and organizations represented at this workshop. Indeed, many in attendance are instrumental to the progress being made in understanding preterm birth. For example, several studies have been undertaken to investigate the link between air pollution and preterm birth, to determine if serum levels of the pesticide 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) and its metabolite 1,1-dichloro-2, 2-bis(chlorophenyl) ethylene (DDE), correlate with preterm births, and to examine drinking water quality for clues as to how chemicals such as polychlorinated biphenyls (PCBs) might influence birth outcomes. All of this work is crucial, although the many promising studies that are providing clues to the environmental aspects of preterm birth will require additional, confirmatory studies.

Our challenge, today and in the foreseeable future, is to remain open to the full range of possibilities for studying preterm birth, to learn from one another, and to share the conviction that eventually we can understand the triggers of this condition. Through this process and the appropriate targeting of resources, we can move forward and take the next step, which will be to fashion public health strategies and clinical interventions that will more fully protect the health of our newborns.

1

Preterm Birth and Its Consequences*

The Roundtable on Environmental Health Sciences, Research, and Medicine is comprised of key stakeholders in environmental health. They meet on a regular basis to discuss areas of mutual concern in a neutral environment. The purpose is to promote discussion, but not to come to consensus. Sometimes, the Roundtable convenes workshops to explore issues in greater depth and facilitate discussion. This is a summary of a workshop convened by the Roundtable on October 2–3, 2001, to look at the issues surrounding the role of the environment in premature birth. The summary has been prepared by the workshop rapporteur as a factual summary of what occurred at the workshop and should not be construed as consensus by the Roundtable or the Institute of Medicine (IOM).

PRETERM BIRTH

More than 400,000 babies are born prematurely each year in the United States. These early births, defined as those occurring before the thirty-seventh week of pregnancy, result in infants who are small and immature—weighing less than 5.5 pounds—and who are at greater risk of death than those born at full term. Indeed the statistics show the struggle that these children face. Disorders relating to short gestation and low birth weight are among 10 leading causes of infant mortality (Hoyert et al., 2001). Earlier estimates suggest that approximate-

*This chapter was prepared from the transcript of the meeting by a rapporteur. The discussions were edited and organized around major themes to provide a more readable summary and to eliminate duplication of topics.

Approximately 35 percent of all expenditures for newborns and approximately 10 percent of all medical expenditures for children are associated with premature birth.

ly 75 percent of all infant deaths in the first month of life occur in premature infants (McCormick, 1985). Premature babies often spend months fighting for survival and struggling to overcome illness, and the impact on families is often long-lasting both emotionally and financially. In economic terms, estimates suggest that in the first year of life alone, a preterm birth costs on average about $59,730 (Rogowski, 1998). Furthermore, approximately 35 percent of all expenditures for newborns and approximately 10 percent of all medical expenditures for children are associated with preterm birth (Lewit et al., 1995). Emotionally, the impact on families is harder to quantify but is reputed to be substantial.

WHAT IS PRETERM BIRTH?

The normal length of pregnancy is 40 weeks (plus or minus 2 weeks) as calculated from first day of the woman's last normal menstrual cycle. A baby born prior to week 37 of gestation is considered premature. However, it is the 1 percent or less of babies who are born at less than 32 weeks and/or who weigh less than 1,000 grams that account for most of the long-term morbidity and mortality observed in premature infants, according to Robert Goldenberg, University of Alabama at Birmingham.

Of all premature babies, approximately 50 percent are born at 35 and 36 weeks of gestation.

Preterm births are classified into two categories: (1) indicated—those deliveries initiated by the clinician for the benefit of either the fetus or the mother, and (2) spontaneous preterm birth—those that follow either spontaneous preterm labor or spontaneous rupture of the membranes. Approximately 20 percent of preterm births are indicated, usually occurring because the mother is severely ill with a life-threatening condition or the fetus shows signs of deterioration and risk of fetal death. Spontaneous preterm labor accounts for the remaining 80 percent. Approximately 30 percent of total preterm births follow spontaneous rupture of the membranes, while the remaining 50 percent follow spontaneous preterm labor, noted Goldenberg. Spontaneous preterm births are often divided into those that occur early and those that occur later. Early preterm births, occurring at less than 30 week's gestation, generally are associated with an intrauterine infection or placental hemorrhage. Later preterm births, which occur be-

Later preterm babies, which occur between 35 and 36 weeks gestation, are, for the most part, not associated with infection, placental hemorrhage, or a specific etiologic factor.

tween 35 and 36 weeks gestation, are, for the most part, not associated with infection, placental hemorrhage, or a specific etiologic factor. Instead, these preterm births appear to happen when the normal mechanisms responsible for term labor take place earlier than usual. Women who have a preterm birth at 35–36 weeks gestation often have an increased number of risk factors—they are underweight, they smoke, and/or they have various psychosocial characteristics—but often no specific precipitating cause is identified for the spontaneous labor or rupture of membranes.

Goldenberg further noted that preterm birth is a heterogeneous entity, with distinctive contributing pathways, which presents a challenge to researchers who study the mechanisms and prevention of preterm birth. Many studies separate spontaneous from medically indicated preterm delivery. Although this is a reasonable strategy, it does not account for the fact that the common indications for medical intervention and early delivery (fetal growth restriction, hypertension) are also independent risk factors for spontaneous preterm birth, stated Goldenberg. Some risk factors may be shared across spontaneous and indicated preterm births, while others are likely to differ. Preterm birth also has been categorized based on clinical presentation (idiopathic preterm labor, preterm rupture of membranes) or on the severity of prematurity as defined by the duration of gestation. More novel approaches to categorization consider the underlying etiologic process—for example, infection/or inflammation and vascular compromise. The challenges related to classification need to be addressed in order to better define end points for epidemiological studies, according to some participants.

INCIDENCE

Preterm birth is not an infrequent event in both the developing and the developed countries. Although the incidence rate is higher in developing countries, the United States and other industrialized countries have seen a steady increase in the incidence of preterm births, noted Goldenberg. From 1981 to 1994, approximately 9 to 10 percent of all births were preterm (see Figure 1.1). This increase continued until 1999 when the incident rate was 11.8 percent (Ventura et al., 2001). For the first time in a decade, a decrease in the number of preterm births was reported for the vital statistics for 2000 (Martin et al., 2002). Most of the increase in preterm birth since 1981 has been among moderately preterm births—those infants born between 32 and 36 weeks of gestation (Ventura et al., 2001). The very preterm birth rate—approximately 1.93 percent (Martin et al., 2002)—has fluctuated little since 1990. Catherine Spong, National

> It is better to have a preterm birth at 34, 35, or 36 weeks where the baby lives, than a stillborn baby at full term.
>
> *Robert Goldenberg*

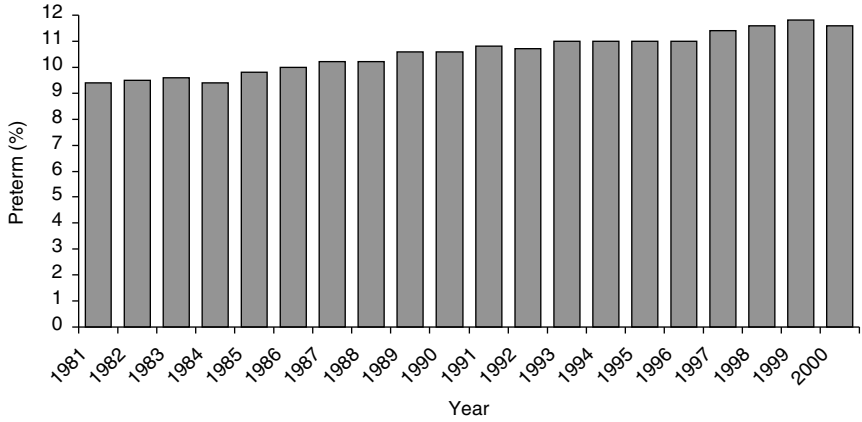

FIGURE 1.1 The incident rate of preterm birth in the United States rose from approximately 9 percent in 1981 to 11.8 percent in 1999. Calendar year 2000 marked the first time in a decade that a decrease in the incidence rate for premature birth was noted. SOURCE: Martin et al., 2002.

Institute of Child Health and Development, suggested that some of the increase is due to the increased use of assisted reproductive technologies; however, predominantly, the increase cannot be explained.

Similarly in Canada, the preterm birth rates also increased during the same period according to Daniel Krewski of the University of Ottawa. He noted that despite the increase, the infant mortality rate has been steadily decreasing at all gestational ages from what it was a decade ago. While this is good news, Goldenberg cautioned that prematurity or reducing prematurity is not the end point. The major goal is to have both healthy babies and mothers. It is better to have a preterm birth at 34, 35, or 36 weeks where the baby lives, than a stillborn baby at full term.

African Americans in the United States have a higher incidence of preterm birth (17.4 percent) than Caucasians (10.4 percent).

Hidden within the statistics is the disparity among populations, according to several participants. African Americans in the United States have a higher incidence of preterm birth (17.4 percent) than Caucasians (10.4 percent). The demographic differences have led some investigators to characterize preterm birth as a disease of poverty. Interestingly, although African Americans have a higher rate of preterm birth, their incident rate has decreased consistently since 1990. During the same period, the preterm birth rate has remained the same for Hispanics, but the preterm birth rate for non-Hispanic whites has shown a steady

increase from 8.5 to 10.4 percent (Martin et al., 2002). The factors that contribute to the variation in the distribution of preterm birth across the U.S. population, particularly the higher incidence for African-American women, have not been determined. According to Goldenberg, more African-American women have bacterial vaginosis, histologic and clinical chorioamnionitis, postpartum endometritis, and genital tract infection, which may be risk factors. Further, some African-American women also may have a low-grade chronic intrauterine infection before or between pregnancies that may be the cause of repeated spontaneous preterm births. This finding appears to be unrelated to the age of onset of sexual activity or number of sexual partners.

LONG-TERM OUTCOMES OF PREMATURE INFANTS

Numerous advances, both technological and therapeutic, in neonatal intensive care have led to the improved survival of premature infants. Prior to 1940, the deaths associated with a preterm birth occurred either at or immediately following birth, according to Maureen Hack, Rainbow Babies and Children's Hospital. Those babies who survived generally were born at 32 weeks of gestation (or later). Since the early 1960s, we have seen a steady increase in the survival rates of premature infants weighing between 1,000 and 1,500 grams and similar, albeit not as high, increases for those infants whose birth weight was well below 1,000 grams. Hack reported that when she started following premature children in the late 1970s, babies born at about 29 weeks' gestation were being treated and some survived. In the 1980s, neonatologists were keeping babies alive at 26 weeks' gestation, and in the 1990s, babies born at 23 or 24 weeks of gestation were surviving. Today, we still see a high percentage of mortality in infants born at less than 26 weeks of gestation; however, those born after 27 weeks of gestation have a greater probability of survival (Figure 1.2).

> Using current methods of neonatal intensive care, we have reached the limits of survival for premature infants.
>
> *Maureen Hack*

Hack speculated, however, that using current methods of neonatal intensive care, we have reached the limits of survival of premature infant, because there has been no significant change in the survival rates in recent years. Not surprisingly, the increase in survival rate of preterm infants has been associated with an increase in neonatal complications, including brain injury and chronic lung disease, which have been most common among the smallest and least mature infants.

Complications of prematurity include respiratory distress syndrome, brain hemorrhage, jaundice, and infections, according to Hack. Other complications

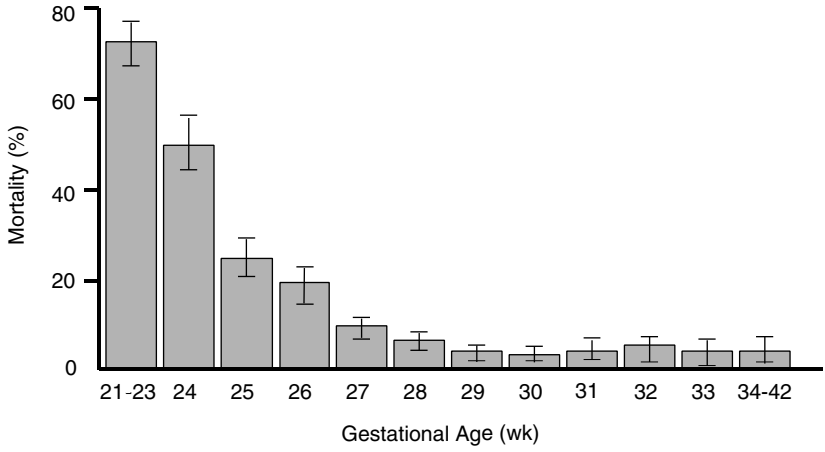

FIGURE 1.2 The percentage of neonatal mortality is inversely related to gestational age. Infants born at or after 27 weeks of gestation have a mortality rate less than 20 percent. SOURCE: Lemons et al., 2001. © 2001 American Academy of Pediatrics. Reprinted with permission.

such as neurodevelopmental disabilities or chronic lung disease are not diagnosed or discernable at birth or shortly there, however, may extend over the course of a lifetime. These impacts have not been fully investigated. The majority of premature babies do not develop neurodevelopmental disabilities as assessed at birth or shortly thereafter; however, many of the impacts become apparent as the infant reaches school age. Overall, premature children require more services beyond routine care throughout childhood, including counseling, occupational therapy, physical therapy, special education, and special school arrangements.

Currently, health care professionals are reporting fewer incidences of blindness due to the increased use of laser and cryotherapy; however, other complications, such as cerebral palsy, have been on the rise for infants born at less than 28 weeks' gestation since 1975 (Figure 1.3). These impairments are inversely related to gestational age and birth weight, such that the younger the gestational age, the greater is the likelihood of an infant having a neurological or a developmental impairment. Predictors of poor neurodevelopmental outcomes include the neonatal complications of prematurity as well as socioenvironmental risk factors. In addition, the same socioenvironmental factors that predispose the fetus to preterm birth usually continue after birth and may have deleterious effects on childhood health and development.

Major neurodevelopmental impairments, including cerebral palsy and mental retardation, that may result from preterm birth can be diagnosed in early

childhood. However, more subtle problems in behavior and functioning (such as poor visual–motor functioning, poor gross motor functioning, reduced math abilities, deficient attention skills, and hyperactivity) may be present at later ages even among children who have no overt neurodevelopmental sequelae. Although respiratory and other health problems tend to diminish during childhood and catch-up growth occurs among many of these children, school functioning problems often persist into adolescence. More preterm than term-born children fail grades, and fewer complete a high school education, noted Hack. Differences in intelligence between normal-term and premature children are evident even among premature children who weighed between 3 and 5 pounds at birth and who did not require neonatal intensive care.

Hack noted that studies by Breslau and colleagues (1994) in Detroit have shown that an increase in IQ test scores, both verbal and performance, accompanies increasing birth weight (Table 1.1).

Little research has been done on the health status of premature infants as they reach adulthood. Hack reported that premature infants displayed less deviant behaviors, less sexual activity, and less drug abuse in a cohort she studied until the individuals reached the age of 20. She further found that the low birth weight population had a lower rate of pregnancy (Hack et al., 2002). Some

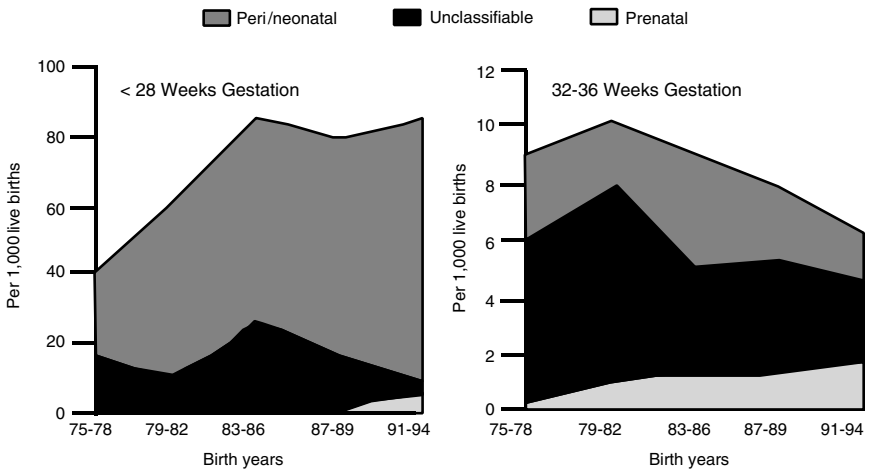

FIGURE 1.3 Since 1975 the prevalence rate of cerebral palsy in infants, whether the result of prenatal, perinatal, or unclassified causes, has been increasing for infants born at less than 28 weeks gestational age compared to infants born at age 32–36 weeks of gestation.
SOURCE: Hagberg et al., 2001. © 2001 Taylor & Francis. Reprinted with permission.

TABLE 1.1 Adjusted WISC-R[a] Scores of Children in Three Low Birth Weight Levels and Normal Birth Weight

Birth Weight (grams)	No. of Subjects	WISC-R IQ		
		Full scale IQ[b]	Verbal IQ[b]	Performance IQ[b]
≤ 1,500	76	95.8 (1.5)	97.5 (1.6)	94.8 (1.6)
1,500–2,000	92	97.8 (1.4)	99.2 (1.5)	96.6 (1.4)
2,000–2,500	300	102.3 (0.8)	102.6 (0.8)	101.5 (0.8)
≥ 2,500	348	105.2 (0.7)	105.6 (0.8)	103.9 (0.7)

[a]WISC-R Wechsler Intelligence Scale for Children-Revised.

[b]Least-square means and standard errors estimated in multiple regression analyses with population site, maternal IQ, maternal education, and race as covariates.

SOURCE: Breslau et al., 1994. © 1994 American Medical Association. Reprinted with permission.

additional research has found that when these low-birth rate women have children, they have a greater probability themselves of giving birth to a low birth weight baby. The results of these studies need additional work in order to better understand the long-term and transgenerational effects of preterm birth.

MEASURING PREDICTORS OF PRETERM BIRTH

Preterm birth is a highly complex process influenced by multiple environmental factors, genetic factors, and gene-environment interactions. In fact, less than half of all preterm births are associated with an identifiable risk factor.

Preterm birth is a highly complex process influenced by multiple environmental factors, genetic factors, and gene–environment interactions. In fact, less than half of all preterm births are associated with an identifiable risk factor. Researchers have used epidemiology as the cornerstone for establishing risk factors and determining intervention strategies for preterm birth. Speakers and participants discussed many of these risk factors, which include biological, individual health behavior, and population factors (Box 1.1). Participants stressed the importance of defining risk factors, not just universally for all populations, but also for individuals or specific subgroups. This may allow the development of more targeted intervention strategies. Presently, the strongest predictors of preterm birth are limited to multiple gestation, prior preterm birth, and African-American ethnicity, according to David Savitz, University of North Carolina School of Public Health. However, research has failed to determine a predominant risk factor or factors, which suggests multiple causal pathways. While most previous studies have focused on

BOX 1.1
Risk Factors Potentially Associated with Preterm Birth as Identified by Speakers and Participants

- African-American ethnicity
- Body size
- Cocaine use
- Infection
- Minimal prenatal care
- Multiple gestation (e.g., twins, triplets)
- Physical exertion
- Prior preterm birth
- Psychological stress
- Short cervixes
- Socioeconomic status
- Tobacco use

socioenvironmental or clinical variables, the role of genetic susceptibility and gene–environment interactions remains largely unexplored (see Chapter 2).

There are distinct issues when researchers characterize preterm birth as a reproductive end point, stated Savitz. He suggested that the idea of dividing preterm birth based on clinical presentation is of potential value but may be misleading in identifying subgroups with differing etiology. Providers often act in practice as though the determinants or risk factors are similar across the spectrum of severity of preterm birth. Just as the consequences of preterm birth vary depending on gestational age, participants considered if the etiologic factors differ and whether subgroups should be considered based on the severity of prematurity. Further, according to Savitz, there is a judgment on the part of the researchers of how inclusive or exclusive to make the outcomes of interest. For example, preterm birth and low birth weight often do not identify the same pregnancies. A high proportion of premature babies are not low birth weight babies; conversely, a high proportion of low birth weight babies are not premature. It is only at the extremes that these two measures predict each other well. As we begin to address these issues from a multidisciplinary approach, we may begin to make further strides in understanding the risk factors and mechanisms underlying preterm birth.

INTERVENTIONS IN PRETERM BIRTH

The goal of any pregnancy is to have labor and delivery occur at an appropriate time to ensure healthy babies and mothers. If premature infants were born with no co-morbidities, the fact that an infant was premature would be of little consequence. However, morbidities do occur in a significant number of preterm births, which has resulted in researchers proposing a number of prevention strategies aimed primarily at spontaneous preterm birth.

Interventions to reduce spontaneous preterm birth are categorized as either general or targeted. General interventions might include providing prenatal care,

improving the general quality of prenatal care, providing across-the-board nutritional supplementation, or providing social support or home visiting. More targeted, experimental interventions may include providing specific aspects of prenatal care—for example, risk screening; nutrition counseling; caloric supplementation; protein supplementation; iron supplementation; labor-inhibiting agents; drug, alcohol, and tobacco cessation programs; bed rest; hydration; and home uterine activity monitoring. However, these types of interventions have rarely been shown to be beneficial in reducing the rate of preterm birth. In fact, there is no evidence that any of the numerous strategies currently used to reduce preterm birth, when applied to various populations, are effective, according to Robert Goldenberg of the University of Alabama at Birmingham. This may be because current intervention strategies are often suggested from risk factors that although associated with preterm birth, may not be directly linked to preterm birth. Some participants suggest that additional research may be needed to better understand the processes underlying preterm birth and to develop better inventions. This section reviews the interventions discussed by various speakers and participants.

There is no evidence that any of the numerous strategies currently used to reduce preterm birth, when applied to various populations, are effective.

Robert Goldenberg

Antibiotics

Infections, such as bacterial vaginosis, syphilis, gonorrhea, and periodontal disease, have all been associated with increased rates of preterm birth and may account for the preterm births of unknown etiology. In the 1980s and 1990s, clinicians began to hypothesize that subclinical infections in either the umbilical cord, the deciduous space, or the amniotic cavity may result in a preterm birth, according to James Roberts, Magee-Women's Research Institute. Romero et al. (1993) cultured amniotic fluid from women in preterm labor. They reported that women with a positive culture were more likely to have a preterm birth, whereas those with a negative culture did not. The mounting evidence of infection as a risk factor suggested that the use of antibiotics may be important for the prevention of preterm birth.

Various attempts at using antibiotics as an intervention for preventing preterm birth have had mixed results. Often antibiotics have been administered to women experiencing preterm labor to prevent or delay delivery. The result of the use of antibiotics in this situation has been inconsistent (see Table 1.2), said Goldenberg, and although two randomized studies, both using metronidazole and ampicillin, suggest that there may be some minimal benefit. Generally, how-

ever, the use of antibiotics alone in women who are in preterm labor has not been successful, and is not likely to have a major impact for women in developed countries.

Bacterial vaginosis is an independent risk factor associated with preterm birth, which is complicated by the fact that many women are asymptomatic. Treating pregnant women with asymptotic bacterial vaginosis with two doses of 2 grams of metronidazole 48 hours apart on two occasions was not effective in reducing preterm birth. However, some studies suggest that antibiotic administration for the treatment of bacterial vaginosis was effective in women who have had a prior preterm birth. Although the use of antibiotics in developing countries has been useful, this administration in developed countries has had mixed results. Since there is an association between preterm birth and infection, it may be possible that, as researchers learn more about underlying mechanisms, specific antibiotics may be useful in some clinical cases.

Prenatal Care

Prenatal care, which involves the provision of social support, home visiting, and nutritional counseling, has been hypothesized to be inversely related to the incidence of preterm birth. However, when studies have examined the data where the amount of prenatal care was increased (e.g., prenatal care was provided to a population that had none, more prenatal care to women who had been receiving little, more prenatal care in a location where only routine care was available), researchers failed to see a reduction in the incidence of preterm birth (Goldenberg and Rouse, 1998). Although prenatal care may not reduce or prevent preterm birth, it does have some benefits. For example, Goldenberg noted that increased prenatal care was effective in decreasing the rate of stillbirths and reducing term mortality in Alabama.

Bed Rest

Bed rest has been used to treat many conditions during pregnancy. It is a recommended treatment for first-, second-, and third-trimester bleeding; prevention of preterm labor in pregnancies with twins, triplets, or higher order multiples; prevention and treatment of preeclampsia; treatment of nonproteinuric hypertension and edema; and treatment of growth retardation. However, randomized studies have shown no improvement in outcomes associated with bed rest in conditions such as first-trimester bleeding; prevention and treatment of preeclampsia; treatment of nonproteinuric hypertension; and treatment of growth retardation, according to Goldenberg. Similarly, randomized studies have failed to show any benefits of bed rest associated with preterm labor (Goldenberg and Rouse, 1998). In the four randomized studies of bed rest as an intervention to reduce preterm delivery in twin studies, no benefit was reported (Harti-

TABLE 1.2 Randomized Controlled Trials of Antibiotics in Women in Preterm Labor with Intact Membranes

Author	Year	Weeks of Gestation	Number
McGregor et al.	1986	<34	17
Morales et al.	1988	21–34	150
Newton et al.	1989	24–34	95
McGregor et al.	1991	_34	103
Newton et al.	1991	24–33	86
McCaul et al.	1992	19–33	40
Romero et al.	1993	24–34	277
Norman et al.	1994	26–34	81
Watts et al.	1994	<34	56
Gordon et al.	1995	24–35	95
Cox et al.	1996	24–34	78
Svare et al.	1997	26–34	110
Oyarzun et al.	1998	22–36	170
Kenyon et al.	2001	24–33	6295

SOURCE: Goldenberg, 2002. © 2002 Lippincott Williams & Wilkins. Reprinted with permission.

kainen-Sorri et al., 1984; Saunders et al., 1985; Crowther et al., 1989; Maclennan et al., 1990).

Nutritional Interventions

Epidemiological studies have found a relationship between women who gain very little weight during pregnancy and the incidence of preterm birth, stated Goldenberg. This suggested that nutritional interventions may help prevent pre-

	Outcomes		
Antibiotics (type)	Decrease in Delay in Delivery	Decrease in Perinatal Preterm Delivery	Mortality and Morbidity
Erythromycin	Yes	No	No
Erythromycin Ampicillin	Yes	Yes	Not Stated
Ampicillin Erythromycin	No	No	No
Clindamycin	Yes	No	No
Ampicillin Sulbactam	No	No	No
Ampicillin	No	No	No
Ampicillin Erythromycin Amoxicillin	No	No	No
Ampicillin Amoxicillin Metronidazole	Yes	No	Yes
Mezlocillin Erythromycin	No	No	No
Ceftizoxime Ampicillin Sulbactam Augmentin	No	No	No
Ampicillin Metronidazole	Yes	Yes	No
Amoxicillin Erythromycin	No	No	No
Erythroymcin Amoxicillin, Clavulanic Acid	No	No	No

mature birth. Currently, researchers have found that the majority of the success has occurred in developing countries where evidence suggests that nutritional intervention has substantially improved pregnancy outcomes, including a reduction in preterm birth. This improved outcome was seen in Gambia, where women worked hard in the field and were nutritionally deprived during certain seasons of the year (Prentice et al., 1983). Similarly nutritional benefits were seen in a randomized trial with an HIV population (Fawzi et al., 1998). However, in developed countries, nutritional counseling and caloric supplementation has had

little or no impact on the rate of preterm births, whereas nutritional supplementation has had mixed results. This may be one area where additional research is needed.

PURPOSE AND TIMING OF THIS WORKSHOP

"Mortality is decreasing primarily because of our ability to intervene, but we've really not made much of an impact at all on morbidity," said Donald R. Mattison of Columbia University. Despite research efforts that are complete or ongoing, we still do not understand the many divergent causes of preterm birth, and therefore we lack the interventions needed to prevent it. Many speakers, however, described research findings suggesting that we may be on the verge of understanding the intervening factors of preterm birth at the molecular, individual, and community levels.

Despite research efforts that are complete or ongoing, we still do not understand the many divergent causes of preterm birth, and therefore we lack the interventions needed to prevent it.

Donald R. Mattison

During this two-day workshop, several speakers suggested that a greater understanding of the factors that constitute normal parturition and labor as well as those that contribute to preterm birth is needed. Chapter 2 of this workshop summary focuses on what is known about the normal mechanisms of labor and delivery that might lead to identification of the causes of preterm birth. Chapter 3 provides an overview of the biological causes of preterm birth. Chapters 4 and 5 explore environmental factors, including the social environment, gene–environment interactions associated with preterm birth, and research protocols used by federal agencies that may detect toxicant effects on gestation length. Finally, Chapter 6 presents workshop participants' proposals for future research directions that are needed to address this major public health concern.

2

Labor and Delivery*

Normal pregnancy is a carefully programmed sequence of events, which can be divided into stages that culminate with labor and delivery (see Figure 2.1). Following fertilization, a series of events is set in motion, with the sequence unfolding in a manner and speed determined by both the genome and the environment—that is, by both nature and nurture. As with most biological processes, the duration of pregnancy is gene influenced, rather than gene determined. With the hope of understanding the mechanisms involved in labor and delivery, and ultimately preterm birth, researchers have spent many years looking for the trigger that starts parturition without success. In the normal pregnancy, there is no trigger for parturition, according to Peter Nathanielsz, Cornell University. "In one very real sense, the only certain signal to parturition is fertilization," meaning that during the stages of pregnancy, there are backup mechanisms and fail-safe devices to ensure a successful pregnancy.

> In the normal pregnancy, there is no trigger for parturition. The only certain signal to parturition is fertilization.
>
> *Peter Nathanielsz*

* This chapter was prepared from the transcript of the meeting by a rapporteur. The discussions were edited and organized around major themes to provide a more readable summary and to eliminate duplication of topics.

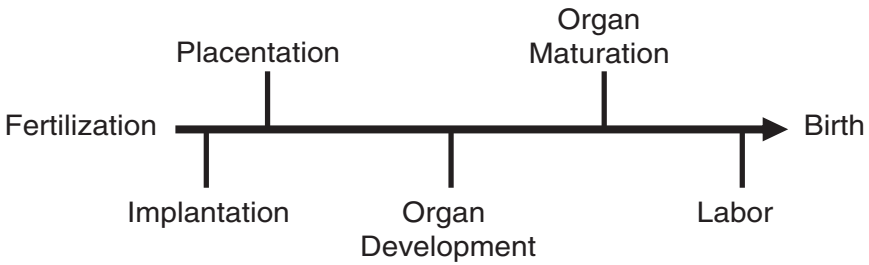

FIGURE 2.1 Normal pregnancy is often divided into various stages, which has led to research into finding a "trigger" or switch resulting in the initiation of labor and delivery. Currently there are no known triggers to move to the next stage, the process is a continuum resulting in delivery of the infant.
SOURCE: Nathanielsz, unpublished, October 3, 2002. Used with permission.

UNDERSTANDING THE SWITCH FROM PREGNANCY TO LABOR AND DELIVERY

Labor and delivery is a multifactorial process that involves fetal, placental, and maternal mechanisms. It involves the recruitment of interactive positive feedback loops and the removal of pregnancy maintenance mechanisms. Changes in these stimulatory and inhibitory mechanisms exhibit critical tissue-specific time relationships to each other. Both fetal and maternal roles are involved in these processes, and it is the interaction of these two roles that determines when birth will occur. Because parturition is a multifactorial system, it is difficult to determine precisely what initiates the cascade of events that leads to delivery. The three indispensable processes involved in normal parturition are (1) a switch in myometrial contractility pattern from contractures to contractions (2) the rupture of the fetal membranes, and (3) the dilation of the cervix.

Myometrium

The onset of labor requires both the activation and the stimulation of the myometrium to generate the intense and coordinated contractions needed to bring about the delivery of the newborn. Animal studies have also provided clues to the mechanisms of myometrial activity or contraction, which has both a fetal and a maternal component. In all mammalian species studied, myometrial activity throughout pregnancy is of the contracture type, exemplified by long-lasting, low-frequency epochs of activity that have a very different temporal and amplitude pattern from contractions (see Figure 2.2).

At labor and delivery, contractures switch to contractions to produce efficient delivery of the fetus. In sheep, this switch occurs once, generally at night, and the ewe proceeds to delivery. In monkey, this switch occurs and augments

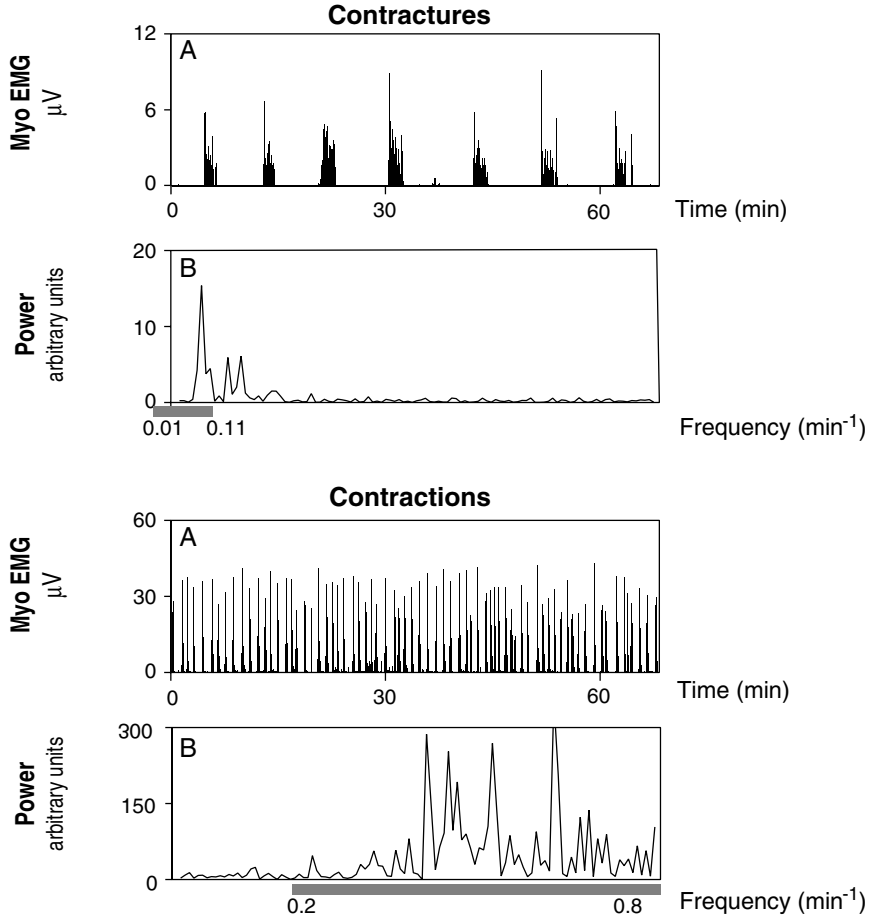

FIGURE 2.2 The amplitude and frequency of uterine activity vary during the course of pregnancy. During pregnancy, myometrial activity is of the contracture type—long-lasting, low-frequency epochs of activity. The activity switches to a high-frequency burst at the onset of labor, which is necessary to deliver the fetus.
SOURCE: Nathanielsz, unpublished, October 3, 2002. Used with permission.

for a few hours each night until delivery occurs after several transitions. By understanding the switch in myometrial activity and the interactions with environmental factors, researchers may better understand mechanism involved in preterm birth. Despite these observations, available evidence suggests that home uterine monitoring is of no value in predicting risk for preterm birth, especially since there are no effective interventions currently.

Genetic Factors

In closely inbred groups of animals maintained under carefully regulated nutritional and environmental conditions, the duration of pregnancy is very homogeneous, said Nathanielsz, suggesting that in such precisely defined environments the onset of labor is precisely regulated by the genome. According to Stephen Lye, University of Toronto, it is the fetal genotype that determines the onset of labor. In cattle bred for longer and shorter gestations, researchers observed that if an embryo from one breed was implanted into the other breed, the length of pregnancy was determined by the fetal genotype. If the cattle were bred so that the fetus had a mixed genotype, then the length of the pregnancy was halfway between the two. Lye presented evidence to suggest that the process of myometrial activation results from the synchronous increased expression of a cassette of genes encoding "contraction-associated proteins," or CAPs, including ion channels which regulate the resting membrane potential of myocytes and hence their excitability; agonist receptors, which enhance myometrial responsiveness to stimulatory hormones; and gap junctions, which provide for enhanced cell–cell communication and therefore synchronization of uterine contractions (see next section).

Maternal Endocrine Factors

Myometrial activation and the onset of labor are ultimately controlled by endocrine and mechanical signals. Progesterone is clearly important for the maintenance of pregnancy, and in most species progesterone levels fall prior to the onset of labor. Animal studies also provide evidence for increased fetal adrenal function in late gestation. In addition, an increase in estrogen production in late gestation has been reported in sheep and various primates, including baboons, rhesus monkeys, and humans (Figure 2.3). These endocrine changes are central to the initiation of labor.

In a series of studies in the pregnant rhesus monkey, Nathanielsz observed that situations resulting in elevated maternal estrogen concentrations in late pregnancy were generally accompanied by a switch in myometrial activity from the contracture to the contraction mode. Such situations included laparotomy and fetal catheterization surgery, hypoglycemia induced by food withdrawal, and the administration of androstenedione to the pregnant monkey. Other investigators have been unable to induce preterm delivery in the pregnant rhesus monkey by the administration of estrogen. Thus, researchers hypothesize that under normal circumstances, estrogen exerts both paracrine effects at its site of generation from androgens and classical endocrine effects.

When Nathanielsz carried out androgen infusion into the pregnant rhesus monkey to ensure that estrogen was produced at its normal locus, labor occurred in the following sequence: a switch of myometrial contractures to contractions,

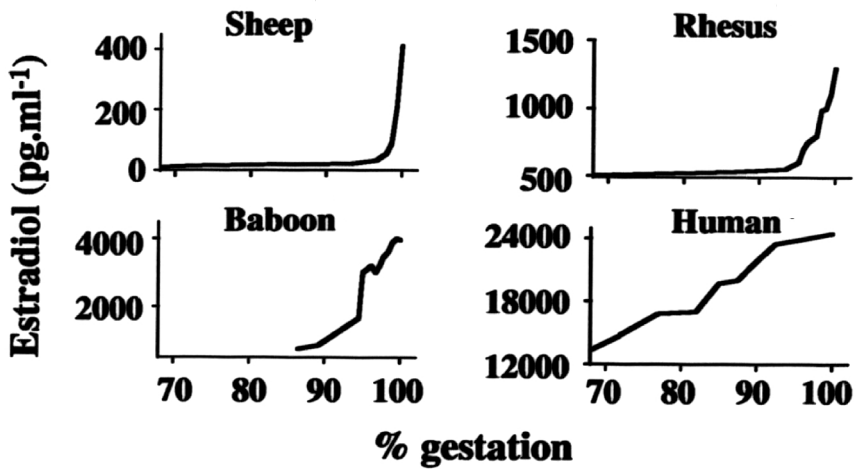

FIGURE 2.3 In most mammalian species studied, the onset of final stages of parturition coincides with a rise in the production of estrogen.
SOURCE: Nathanielsz, unpublished, October 3, 2002. Used with permission.

rupture of the fetal membranes, dilation of the cervix, and delivery of live young. In this experimental paradigm, the switch is prevented if aromatase inhibitors are administered with the androgen to inhibit estrogen production. Although the infusion of androgen was continuous and maternal plasma estrogen concentration was elevated throughout the day, the switch from contractures to contractions occurred only around the hour of darkness, suggesting a role for oxytocin (Mecenas et al., 1996). Maternal plasma oxytocin concentrations in late gestation show a pronounced 24-hour rhythm, with a peak in the early hours of the evening, coincident with the switch from contractures to contractions. A similar rhythm has been shown in pregnant women in late gestation. Further, oxytocin antagonists such as Atosiban will inhibit both the normal-term switch from contractures to contractions and the transition that is produced (prematurely) by androgen infusion.

> The rise in estrogen is the central key for normal labor and delivery, a context within which it becomes interesting to look at environmental factors and environmental disruptions, including stress hormones.

The action of estrogen is complex, but one site of regulation is the expression of CAP genes. As discussed in the previous section, CAP genes are involved in the onset of labor and their expression increases as myometrial activation occurs. Lye suggested that estrogen increases the expression of CAP genes,

while progesterone suppresses their expression. The mechanisms by which these steroids act remain to be determined, although evidence suggests that specific transcription factors contribute to the activation of CAP genes. Interestingly, in humans, progesterone levels do not decrease at term, suggesting that another mechanism exists in humans to block progesterone signaling. This would in essence induce a functional withdrawal of progesterone, permitting activation of CAP genes, myometrial activation, and the onset of labor. The rise in estrogen is the central key for normal labor and delivery, a context within which it becomes interesting to look at environmental factors and environmental disruptors, including stress hormones.

Fetal Endocrine Factors

The fetal endocrine system plays a crucial role in the initiation of labor and delivery. In classic experiments in animal physiology, Liggins found that interruption of the fetal hypothalamic–pituitary–adrenal (HPA) axis at any level prolongs pregnancy in sheep. This occurred if the fetal adrenal glands or the pituitary were removed, or if a wax plate was placed between the hypothalamus and the pituitary (see Figure 2.4).

Conversely, it has been found that infusion of adrenocorticotropic (ACTH) hormone or cortisol to the ovine fetus will shorten the length of pregnancy. These findings have led some to believe that the fetus determines the length of ovine pregnancy. The endocrine pathway involves activation of the fetal HPA axis leading to increased synthesis and release of cortisol from the fetal adrenal gland. Cortisol in turn induces the expression of PGHS-2, the type 2 isomer of prostaglandin H synthase, in the placenta, which leads to the production of fetal prostaglandin E_2 (PGE_2). As PGE_2 levels rise, an induction of cytochrome P450C17 activity occurs, which ultimately leads to the metabolism of maternal progesterone to estrogen.

The increased production of prostaglandins, which also can stimulate the myometrium, coincides with the end of gestation. William Gibb of the University of Ottawa described work in experimental animals in which a decrease in the concentration of progesterone in the maternal plasma has been observed prior to the onset of labor. In parturition, PGHS-2 is particularly important in this pathway, and its expression has been found to increase in term and preterm labor. In fetal sheep, increased cortisol regulates PGHS-2 expression in the placenta in an estrogen-independent manner, resulting in increased levels of PGE_2 in fetal circulation. Later increases in maternal uterine expression of PGHS-2 require estrogen and lead to increased concentrations of $PGF_{2\alpha}$ in the maternal circulation. In women, the fetal membranes surrounding the amniotic cavity are thought to be an important source of prostaglandins involved in parturition. Formation of pros-

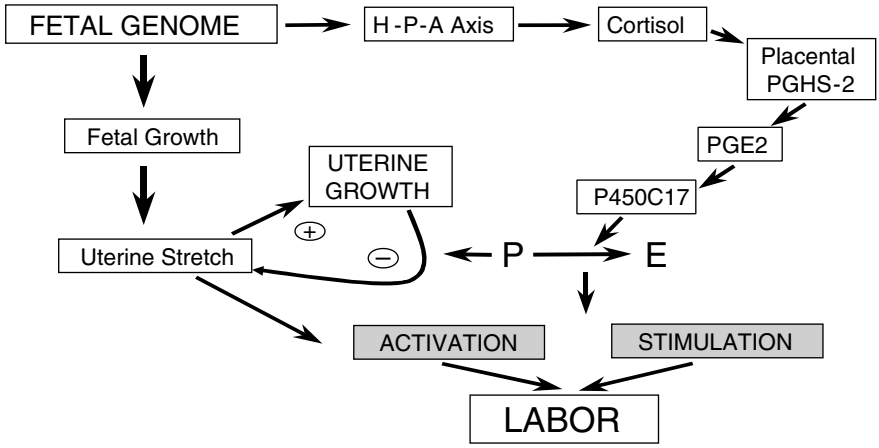

FIGURE 2.4 The fetal genome stimulates labor through two pathways: the endocrine system and uterine stretch. The fetal adrenal gland releases cortisol, which through a molecular pathway results in a rise of cytochrome P450C17. The stimulation of cytochrome P450C17 shifts the hormonal balance from progesterone to estrogen, a key step for the initiation of labor. The second pathway leading to the activation of labor is fetal growth. As the fetus grows, the uterus stretches. In the presence of progesterone, the uterus is allowed to increase the size of the myometrial cells to accommodate the growing fetus. At the end of pregnancy with the shift toward estrogen, the inhibitory effects of progesterone, and resulting in stimulation of labor.
SOURCE: S. Lye, unpublished. Reprinted with permission.

taglandins in these tissues is regulated by paracrine and autocrine mechanisms. Thus, cortisol can contribute to increased prostaglandin production in fetal tissues through upregulation of PGHS-2 and downregulation of 15 hydroxyprostaglandin-dehydrogenase (PGDH), the principal enzyme involved in the metabolism of prostaglandins. The effect of cortisol on chronic expression of PGDH reverses a tonic stimulatory effect of progesterone and likely occurs through the glucocorticoid receptor. The 11β-hydroxysteroid dehydrogenase enzyme appears to be important in regulating the effect of cortisol on prostaglandin metabolism in the placenta and chorion. In turn, this enzyme is upregulated by the fetal membranes by prostaglandins, forming a feedback loop leading to increased prostaglandin production. Cortisol also increases expression of placental corticotropin-releasing hormone, which can in turn increase prostaglandin production. Other agents such as pro-inflammatory cytokines similarly upregulate PGHS-2 and decrease expression of PGDH, indicating the presence of several mechanisms by which labor at term or preterm may be initiated.

Mechanical Factors

Although endocrine pathways are necessary for the initiation of labor, they are not sufficient, according to Lye. Mechanical signals are also required. Previous work has shown that the stretch of the uterus could induce an increase in gap junction production (see below) that may be related to the onset of labor. Fetal growth puts tension on the wall of the uterus, and it has been hypothesized that this tension could activate the same genes within the myometrium as the endocrine pathway. Rats have two uterine horns and can be manipulated such that they are pregnant only in one horn (the other horn is empty). At the time of labor, the endocrine changes occur as normal; however, the labor genes are turned on only in the horn with fetuses. The genes in the empty horn were not turned on even though the empty horn was exposed to the endocrine changes, reported Lye, but if a tube was inserted in the empty horn to stretch the muscle, then all the labor genes were turned on.

> Although endocrine pathways are necessary for the initiation of labor, they are not sufficient. Mechanical signals are also required.
>
> *Stephen Lye*

Interestingly, if stretch activates genes that cause labor, the question remains why animals don't trigger labor as the fetus grows. Lye suggested that it is the presence of progesterone that is a preventive mechanism. As the fetus grows, the stretch in the presence of progesterone causes an increase in the size of the myometrial cells. This increases the growth of the uterus, which results in less tension being applied as a result of fetal growth. It is a protective mechanism that allows the fetus to grow without inducing preterm labor, and it occurs as long as progesterone is present.

Molecular Mechanisms and Cellular Signaling Pathways Associated with Parturition

During parturition, maternal and fetal compartments express the signaling pathways and produce the molecules that stimulate the myometrium to contract. The regulation of myometrial contraction is of overriding importance for the maintenance of pregnancy and for parturition, and understanding this regulation involves delineating the pathways that control contraction and relaxation and defining their interaction. The cellular mechanisms regulating uterine quiescence, the switch to contractivity, and the contractivity itself are complex and constitute an area of ongoing research. The speakers summarized some of the key pathways, receptors, and molecules that may be important.

Cellular Depolarization

The fundamental mechanism that drives uterine contraction is the smooth muscle cells of the myometrium. The myometrium has a number of pacemaker cells that propagate electrical signals to other myometrial cells to produce a forceful contraction. The rhythmic electrical firings are grouped together in bursts, which dictate the contraction patterns of the uterus. The frequency of these bursts dictates the frequency of contractions, while the duration of the bursts dictates this duration. The magnitude of contraction is dependent on the extent of propagation of these electrical signals throughout the uterus to recruit additional smooth muscle cells.

In order to have these smooth, coordinate muscle contractions, the myometrial cells must have rhythmic coupling to provide the contractility necessary for delivery. One way in which this occurs is through gap junctions, which allow the cytoplasm from one cell to be contiguous with the cytoplasm of a second cell. Thus, the channel provides a low-resistance contact between the cells and allows current to spread with minimal delay between cells. More than 20 years ago, Garfield measured the number of gap junctions at various stages of pregnancy and parturition. During pregnancy when the uterus is in a stage of relaxation, there are few gap junctions. They begin to increase in numbers prior to delivery, peak during delivery, and decline thereafter. They play a necessary role for normal labor and delivery in all species, including humans, which have been studied. If the gap junctions form early, preterm delivery results, and if their formation is blocked, delivery will not occur.

Cellular Pathways

Prior to parturition, a transition period occurs during which a series of events prepare the myometrium to respond to contractant signals and to be less responsive to relaxant signals (Table 2.1), said Barbara Sanborn of the University of Texas Medical School, Houston. This requires conformational changes in the actin and myosin molecules, which result in a shortening of the myocytes. Calcium is a key mediator in this process, acting through the calcium—calmodulin and myosin light chain kinase pathway (Figure 2.5).

The pathway to myometrial contraction begins with an increase in intracellular calcium via calcium channels in the cellular membrane or from intracellular calcium stores in the endoplasmic reticulum, which activates calmodulin. The calcium–calmodulin complex then binds to the myosin light chain kinase (MLCK), where it induces a conformational change in MLCK. This conformation change is necessary for the activation of actin and myosin through phosphorylation by MLCK. The calcium concentration is then lowered from the intracellular space by calcium pumps on the extracellular membrane or the endoplasmic reticulum, which returns the cells to homeostasis.

TABLE 2.1 Myometrial Signaling Components in Late Pregnancy

Contraction	Relaxation
Oxytocin receptors ↑	β-adrenergic receptors ↓
α_1-adrenergic receptors ↑	CGRP receptors ↓
$G\alpha_q$ ↑	$G\alpha_i$ ↑, $G\alpha_s$ ↓
PLCb, no change or ↑	Adenylate cyclase, no change or ↓
Inhibition of PLC by PKA ↓	↑ PDE4-2B

CGRP = calcitonin gene-related peptide;
$G\alpha_q$,$G\alpha_s$, $G\alpha_i$ = variants of the G-protein alpha subunit;
PDE4-2B = a type of phosphodiesterase;
PKA = protein kinase A;
PLC = phospholipase C.

SOURCE: B. Sanborn, unpublished. Reprinted with permission.

Although calcium–calmodulin mediates the major pathway associated with uterine contractions, there are opportunities for other pathways to influence the activities of various enzymes. The uterine relaxant pathways involve activation of cGMP cyclic guanosine 5′-monophosphate) and cAMP(cyclic adenosine 5′monophosphate), which in turn activate protein kinase G (PKG) and protein kinase A (PKA), respectively. PKG and PKA phosphorylate several proteins associated with promoting relaxation. They phosphorylate C (PLC), which decreases activation by GTP phospholipase (guanosine 5′-triphosphate)-binding proteins. In addition, circulating hormones such as oxytocin and prostaglandins increase intracellular calcium in part through the PLC/–IP_3 (inositol 1, 4, 5-triphosphate) pathway.

The A-kinase anchoring proteins (AKAPs) are critical to PKA regulation. AKAP 9/150 brings PKA and PP2B (protein phosphatase 2B; the phosphatase that dephosphorylates PKA and other molecules) into close proximity with the PLC/–IP_3 pathway. In fact, there is a switch in the ratio of PKA/–PP2B bound to AKAP 150 in myometrial plasma membrane between days 19 and 21 of gestation in the rat (see Figure 2.6). This switch, in essence, upregulates the effectiveness of the PLC/–IP_3 pathway, while downregulating one of the feedback pathways. Thus, the PLC/–IP_3 pathway is more poised to respond to a signal with an elevation of calcium. It is not yet clear if similar changes in the association of PKA with a plasma membrane AKAP are seen in humans, sheep, and baboons during gestation.

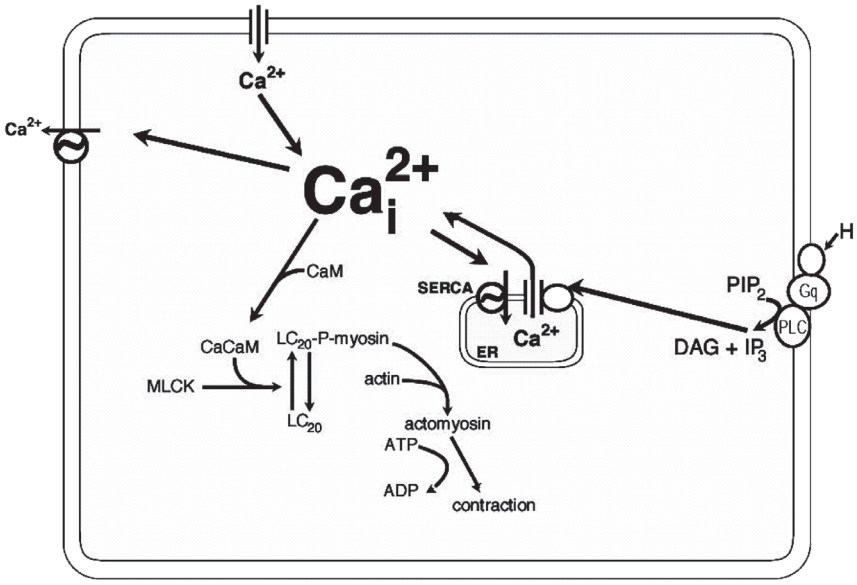

FIGURE 2.5 The rise in intracellular calcium via release from internal stores or through Ca^{2+} channels plays a central role in producing contraction and the initiation of labor. CaCaM = calcium–calmodulin complex; CaM = calmodulin; DAG = diacylglycerol; IP3 = inositol 1,4,5-trisphosphate; LC20 = myosin light chain; MLCK = mysoin light chain kinas; PIP2 = phosphatidylinositol bisphosphate; SERCA = sarcoendoplasmic reticulum Ca^{2+}–adenosine triphosphatases (ATPases).
SOURCE: B. Sanborn, unpublished. Reprinted with permission.

Nitric Oxide

Nitric oxide (NO) exists as a highly reactive free radical in the gas or solution form and is highly diffusible—that is, there is no need for a receptor or transporter to diffuse it. It was first discovered in 1972, but rose to prominence in the 1990s as a mediator of short-term physiological function. Researchers suggest that it may be involved in blood pressure regulation, male impotence, learning and memory, stroke, and so forth. In 1993, Yallampalli and colleagues, at the University of Texas Medical Branch in Galveston reported the presence of the NO system in the uteri of a variety of animals (including humans), suggesting that the endogenous NO synthesized in the uterus could play a role in uterine quiescence during pregnancy.

Several studies have reported increases in NO synthesis in the uterus and in NO-induced uterine relaxation during pregnancy. Both NO synthesis and uterine sensitivity to NO are substantially reduced at term, indicating a role for NO in uterine quiescence during pregnancy and labor (Figure 2.7).

The extent to which uterine NO is necessary for maintaining uterine quies-

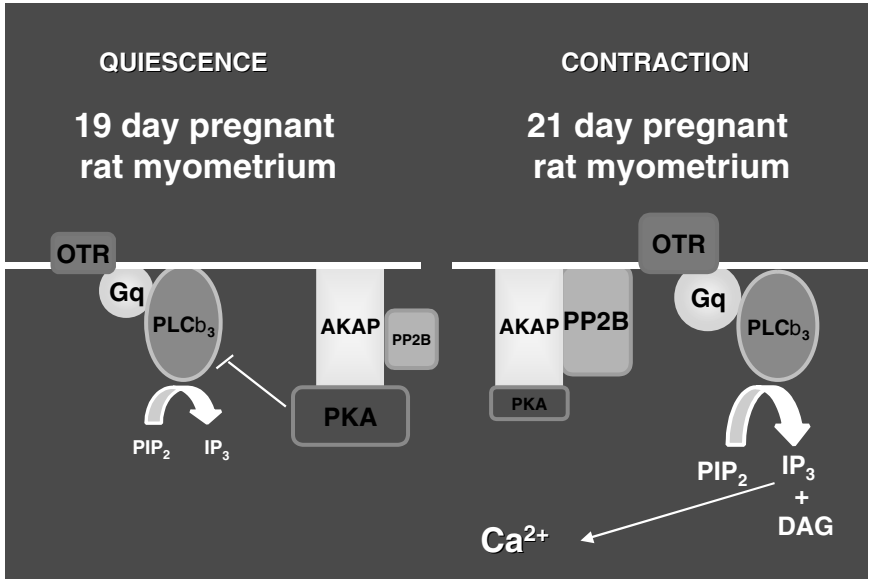

FIGURE 2.6 The A-kinase anchoring protein (AKAP) plays a pivotal role in the shift of the uterus from quiescence to contraction. In the rat between days 19 and 21, there is a decrease in the ratio of bound protein kinase A (PKA) to protein phosphatase 2B (PP2B). The change in ratio results in upregulation of the IP_3 pathway to generate an increase in intracellular calcium.
SOURCE: B. Sanborn, unpublished. Reprinted with permission.

cence during normal pregnancy is under investigation. Studies in the rat and sheep indicate that inhibition of NO synthesis prior to term does not result in parturition; however, in mice, parturition occurs. Moreover, uterine NO synthesis has been shown to be regulated by estradiol and progesterone, key hormones in pregnancy. Progesterone not only enhances NO synthase expression and NO synthesis in the rat uterus but also enhances NO-induced uterine relaxation. On the other hand, antiprogesterones decrease NO synthase enzymes, inhibit NO synthesis, and reduce uterine relaxation responsiveness to NO. Yallampalli suggested that during pregnancy there is a significant increase in NO production driven by the elevated progesterone/estrogen ratio, which switches during normal-term labor. Thus, NO might interact with other uterotonins in the maintenance of uterine quiescence during pregnancy.

Calcitonin Gene-Related Peptide

The calcitonin gene-related peptide (CGRP) also acts through the relaxant pathway. This peptide is synthesized and transferred to many organs, such as the

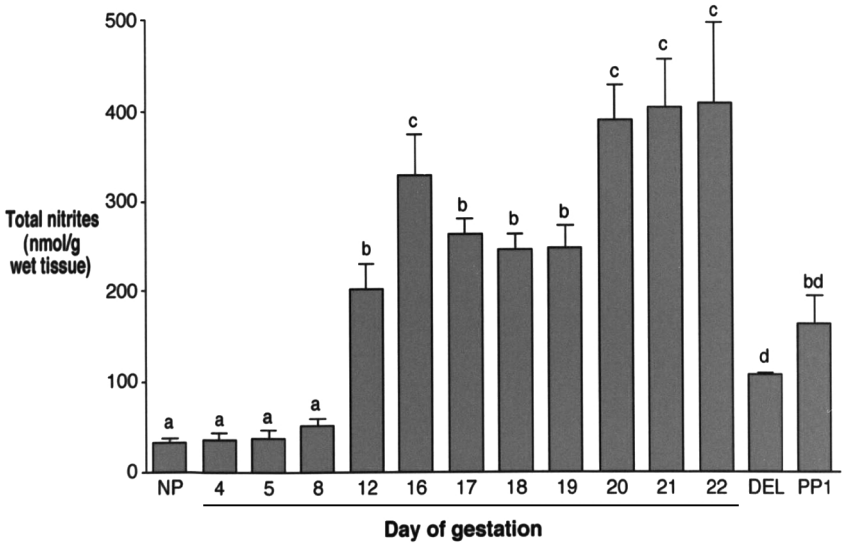

FIGURE 2.7 The role of nitric oxide in maintenance of the rat pregnancy is still under investigation. The concentration of NO rises during pregnancy, but decreases at the onset of labor and delivery. This suggests that NO concentration may be important for maintaining quiescence.
SOURCE: Yallampalli et al., 1998. © 1998 Society of Gynecologic Investigation. Reprinted with permission.

uterus, and to the vasculature. Yallampalli's research has shown that the level of CGRP is increased during pregnancy and falls at the end of gestation, suggesting that the CGRP levels follow relaxation (Figure 2.8). He has concluded that (1) CGRP inhibits both spontaneous and induced contractility of uterus in both rat and human, (2) sensitivity to CGRP is increased with pregnancy and decreased at term, and (3) CGRP binding to myometrium is increased with pregnancy and decreased with labor. Moreover, CGRP receptor levels in myometrium parallel CGRP binding and relaxation responses.

Rupture of the Fetal Membrane and Cervical Ripening

The ripening of the cervix and the rupture of the fetal membranes are both necessary for labor and delivery to occur. In the cervix and fetal membranes, the preparatory step during term delivery is temporally different from that of the uterus, indicating separate control systems.

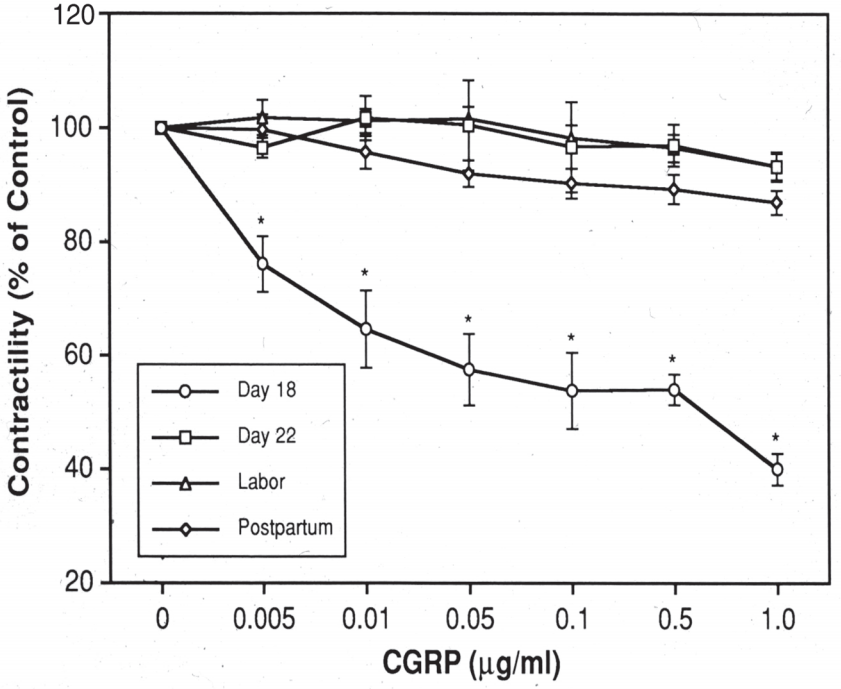

FIGURE 2.8 The calcitonin gene-related peptide (CGRP) may be important for maintaining uterine quiescence. In the rat prior to delivery, the uterus is sensitive to CGRP which has a dose-dependent effect on uterine contractility. As labor and delivery near, CGRP has little effect on contractility as the uterus changes its sensitivity to the peptide. SOURCE: Dong et al., 1998. Reprinted with permission.

Cervical Ripening

To prepare for labor and delivery, the cervix, which is closed and rigid throughout most of pregnancy, must soften and dilate, according to Robert Garfield, University of Texas Medical Branch. This occurs independently of uterine contractivity and is necessary for an uncomplicated delivery. There are three phases to cervical ripening: softening, effacement, and dilation. The softening phase is chronic, while the effacement and dilation phases are acute—occurring immediately before delivery.

Although there are a number of smooth muscle cells, fibroblasts, and mast cells in the cervix, it is primarily comprised of collagen-type connective tissue. At the time of parturition, the breakdown in the cervical matrix coincides with a decrease in the amount of collagen and a subsequent increase in the water content. Further it is the change in type I collagen from an insoluble to a soluble

form that is crucial to ripening. The two forms of collagen have a different fluorescent pattern that can be monitored by a collascope. Garfield reported that in humans and rats there is a decline in the fluorescent pattern of the cervix as parturition nears, which corresponds to the change in type I collagen. Further, they were able to show in patients that when the fluorescent patterns decrease due to changes of collagen content, the cervix softens and the patient will deliver preterm.

The biochemical pathway resulting in the breakdown in the cervical matrix has been extensively studied (see Uldbjerg 1989; Leppert,1995, for review). These pathways can be activated by a number of external compounds and provide possible sites of interactions for environmental factors to play a role. The activation of prostaglandins and the subsequent activation of compounds such as NO constitute an area of ongoing research. The role of NO appears to have different effects in activating the myometrium or softening the cervix, according to Garfield. NO levels are elevated in the uterus (as discussed previously) during pregnancy and are associated with uterine quiescence. At the onset of labor, production of NO decreases, resulting in an increase in uterine contractility. In contrast, NO production increases in the cervix as labor begins and appears to be involved in softening of the cervix. Similar results (cervical softening) have been obtained by locally applying NO agonists to the cervix.

Fetal Membranes

The fetal membranes, which surround the amniotic cavity, are composed of two layers: the amnion and the chorion. The primary function of the membranes is to retain the amniotic fluid, to secrete substances into the fluid and the uterus, and to protect the developing fetus from infection. Rupture of the membranes is the third event necessary for delivery, and it occurs shortly before parturition begins. Preterm rupture of the membranes occurs in approximately 1 percent of all pregnancies and is associated with 30–40 percent of all preterm deliveries.

Preterm rupture of the membranes occurs in approximately 1 percent of 23 pregnancies and is associated with 30–40 percent of all preterm deliveries.

Fetal fibronectin is a protein, which is part of the extracellular matrix between the membranes and the decidua. It is used as a marker of impending labor, especially preterm birth. When an inflammation or breakdown of the extracellular matrix occurs, fibronectin leaks down into the cervix and the vagina. Goldenberg stated that women in early preterm labor with a negative fetal fibronectin test have a less than 1 percent chance of delivering in the subsequent two weeks.

3

Preterm Birth—Brief Summary of Biological Pathways*

Although researchers have been able to determine a number of potential risk factors for preterm birth, the fact remains that fewer than 50 percent of all preterm births are associated with an identifiable risk factor. Many participants discussed opportunities where convergence can occur in various pathways to begin the process of preterm labor, including biological mechanisms, environmental influences, and gene–environment interactions that may play a role. By understanding the normal labor and delivery process and potential mechanisms of preterm labor, additional progress could be made according to many individuals.

BIOLOGICAL CAUSES AND MECHANISMS OF PRETERM LABOR

Three broad categories of biological factors have been suggested to play a role in the onset of preterm birth: abnormality of the biological clock, abnormal implantation and infection and inflammation.. These current theories provide multiple sites at which environmental factors may influence biological factors to induce preterm birth.

* This chapter was prepared from the transcript of the meeting by a rapporteur. The discussions were edited and organized around major themes to provide a more readable summary and to eliminate duplication of topics.

Aberrant Fetal Clock

The idea that preterm delivery is due to an aberrant fetal clock is one theory that has been around for a long time, according to some participants. This theory developed because the fetus stimulates the onset of normal labor, probably through endocrine and paracrine mechanisms, but also through mechanical stimulation, said James Roberts of Magee-Women's Research Institute. As discussed Chapter 2, as the fetus grows there is an increase in uterine size. The uterus is usually able to accommodate the growing fetus as long as progesterone is present. A recognized cause of preterm birth is, in fact, uterine distension with multiple gestations, or polyhydramnios. In this case, the symptom certainly occurs earlier, and we have an aberration of that particular component of the clock starting labor. However, Roberts cautioned that most people would not agree that the majority of preterm labor is just term labor occurring early. He suggested that we currently do not know enough about preterm labor to assume any hypothesis.

Abnormal Implantation

Implantation occurs approximately five days after fertilization. In order to supply the placenta and ultimately the fetus, a number of changes in the vascular system of the uterus occur. In the normal pregnancy, there is striking remodeling of the vessels that supply the intravillous space, resulting in a marked increase in luminal diameter and the loss of smooth muscle and elastic components of the vessel wall. The endothelial lining is replaced at least in part by trophoblastic cells that have been modified to express a vascular phenotype.

Roberts noted that under certain conditions, this normal remodeling and the subsequent increase in blood supply to the placenta do not occur—for example, in preeclampsia, growth restriction, and one-third of preterm birth. This suggests that preterm birth may be one of a number of implantation diseases, and these various conditions may have a number of similarities. For example, abnormal implantation is more likely to occur in a first pregnancy. In a recent study, Roberts found an additional link between preterm birth and preeclampsia. The iron storage protein ferritin—a measure of serum iron levels—is linked to both acute and chronic infections. In both preeclamptic pregnancy and preterm birth, ferritin is increased in the blood of these women long before they deliver preterm. This suggests an increase in inflammatory activation in early delivery and preeclampsia (see cytokines below).

Preterm Birth and Infection

In the 1980s and 1990s researchers suggested that subclinical infections in either the choriodecidual space or the amniotic cavity may lead to preterm birth. Interestingly, according to Robert Goldenberg, up to 80 percent of early preterm

births are associated with intrauterine infection that precedes the rupture of the fetal membrane.

In a classic study, Romero et al.(1993) examined the amniotic fluid of women experiencing preterm labor. Although approximately 70 percent of those diagnosed with preterm labor will go to term, Romero found that those women who delivered preterm had a positive culture of the amniotic fluid. Conversely, those with negative cultures did not, according to Roberts. Often, this intrauterine infection is linked to the presence of bacterial vaginosis, which in nearly 20 studies is associated with an approximately twofold increased risk of preterm birth.

More recently, an interesting association between subclinical infections—such as periodontal disease—and preterm birth has been suggested. Goldenberg reported that in a study at his institution, 83 percent of the spontaneous preterm births weighing less than 1,000 grams were associated with bacteria in the fetal membranes prior to membrane rupture, indicating that a chronic intrauterine infection with relatively low-virulence organisms such as ureaplasma, mycoplasma, and bacteroides is associated with most early preterm births.

Cytokines: A Common Pathway?

The mechanisms by which infections are proposed to lead to preterm birth has been an area of ongoing investigation. Initially, researchers thought that certain bacteria had the capacity to activate phospholipases or had their own phospholipases. It was hypothesized that phopholipase A_2 generated the release of arachidonic acid and prostaglandins, which ultimately resulted in uterine contractions. However, according to Roberts, newer research is focused on the ability of infections to turn inflammatory mediators of cytokines, with the secondary effects of the cytokines to mediate many of the effects of the infection.

The possible role of cytokines in mediating the effects of infection raises the question of whether it is possible that activation of cytokines, in the absence of infection, could be a cause of preterm birth. Roberts discussed some of the evidence currently accumulated. Cytokines obtained from amniotic fluid are actually better predictors of chorioamnionitis in the chorioamnion than is a positive culture. This may be because cultures are more difficult to perform than interleukin-6 (IL-6) determinations; however, Roberts suggested that a number of epidemiological factors and mechanistic factors that are associated with preterm birth are also associated with increased cytokines production. For example, hypoxia (as a result of abnormal implantation), immune responses, stress (through corticotrophin-releasing hormone), and decidual bleeding (through the thrombin receptor)—all of which have been hypothesized as playing a role in premature birth—can lead to an increased production of cytokines. This might occur through the formation of the enzyme cyclooxygenase-2, which stimulates prostaglandins. As discussed in chapter 2, prostaglandins are thought to be one cellular pathway

that activates the myometrium. A second hypothesis is that through stimulation of the formation of metalloproteinases, cytokines degrade proteins, which will lead to preterm rupture of membranes. Presently, there are a number of unanswered questions regarding this hypothesis. Some participants suggested that this is an area in need of additional research.

4

Preterm Birth—Gene–Environment Interactions*

The environment in which we live, play, and work has long been suspected to play a major role in determining the health of individuals and animals. Using a broader definition of environmental health, workshop participants addressed some of the research that provides a direct linkage between environmental toxicants and preterm birth. One observation that has led to many studies of the role of the fetus in initiating labor and an early clue to disruption of normal pregnancy is the ingestion of *Veratrum californicum*, a flowering plant found commonly in Rocky Mountain meadows. It is known to contain a toxic compound that produces malformations in the sheep fetus when ingested during pregnancy. According to Nathanielsz, the fetus is severely deformed, with a large central cyclopian eye, and macroglossia (the tongue protrudes from the mouth because it is too large), and the base of the brain is disorganized. Further the fetus continues to grow and, at term, onset of labor of labor does not occur (see Figure 4.1). Observations such as this have led researchers to ask how the environment may interact with the normal processes of pregnancy to interfere with labor and delivery. This chapter summarizes some of the research and research programs that may shed light on environmental causes of preterm birth.

* This chapter was prepared from the transcript of the meeting by a rapporteur. The discussions were edited and organized around major themes to provide a more readable summary and to eliminate duplication of topics.

ENVIRONMENTAL FACTORS ASSOCIATED WITH PRETERM BIRTH

Adverse effects on the developing organism, which may be detected at any point in the life span of the organism, may result from exposure, prior to conception, of either parent, during prenatal development, or exposure postnatally up to the time of sexual maturation. Researchers are only beginning to identify those exposures and levels of exposures that affect reproductive outcome. According to Matthew Longnecker, National Institute of Environmental Health Sciences (NIEHS), the environmental risk factors for other reproductive outcomes can differ from those for preterm birth.

The potential risk factors studied can be divided into a number of broad categories, including: occupation or occupational exposures, air pollutants, exposure to POPs (persistent organic pollutants), exposure to DDE (1,1-dichloro-2, 2-bis(chlorophenyl) ethylene) a metabolite of DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT), and PCBs (polychlorinated biphenyls), metals (e.g., lead, arsenic), water disinfection byproducts, and video display terminals.

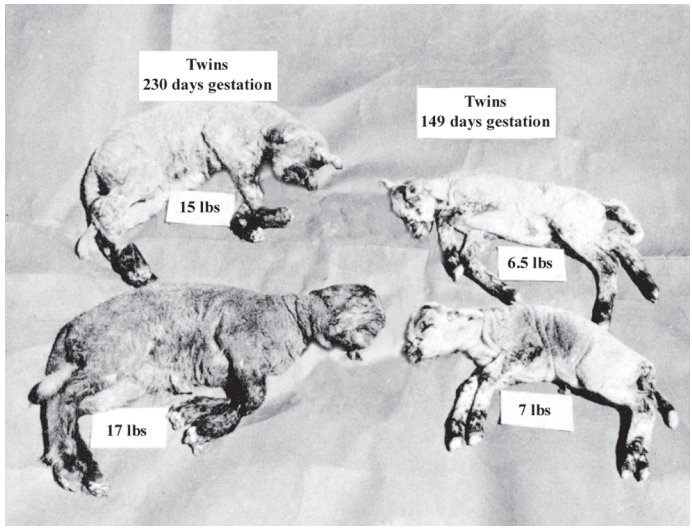

FIGURE 4.1 Sheep that ingest the flowering plant *Veratrum californicum* during pregnancy prolong the length of gestation. A pair of twin sheep delivered at 230 days of gestation by cesarean section (left), approximately 80 days longer than the normal pregnancy (right). This would be equivalent to a woman being pregnant for 15 months and not showing any indication of entering the stages of labor.
SOURCE: Binns et al., 1964. © 1964 Annals of the New York Academy of Sciences. Reprinted with permission.

Among the exposures linked to occupation and occupational exposures, factors that were included in the discussions at the workshop were for those exposures in which the relative risk of preterm birth was 1.5 or greater or the finding was statistically significant, and the finding was reported in two or more studies. Longnecker noted a difference of relative risk between women and men. Women who were employed in metal working, electrical occupations, janitorial work, food service, and textiles had a higher relative risk. In contrast, men who were working in food service or textiles had some association. This sparked some discussion among participants, who wondered why food service as an occupation had a higher relative risk. Some participants noted that occupations and titles often are not clear and additional work would be needed to answer the questions surrounding these observations. There was a further difference between females and males regarding occupational exposures. Longnecker noted that solvents were the most common risk factor for maternal occupational exposures while the most common risk factors for males were pesticide exposures.

The second category of exposure that has been studied in relation to premature birth is air pollutants. These can be divided into two types: tobacco-related (e.g., passive smoking, environmental tobacco smoke) and traditional air pollutants (e.g., particulates, sulfur). The evidence on direct tobacco use is discussed in Chapter 5; however, Longnecker reported that studies showed that a moderately elevated risk of premature birth was associated with direct smoking. It was interesting that the relative risks for environmental tobacco smoke tended to be higher than those for direct smoking. Researchers are beginning to study the relationship between traditional air pollutants and premature birth. Longnecker noted that of the three studies that had ecologic time-series designs and some degree of individual-level data, in two there was an increase in preterm delivery associated with exposure to sulfur dioxide, which comes primarily from coal burning. Even at lower levels of particulates, the relative risk associated with particles less than 10 microns in diameter was high as measured in one study in Los Angeles. Two other studies also supported an adverse effect of total suspended particles. The time of exposure during pregnancy may be important for causing preterm birth. In one study, researchers found that if exposures occurred in the first trimester, the relative risks were greater. Longnecker noted that many questions remain. For example, data on sulfur dioxide in the United States are lacking. They don't burn coal in Los Angeles, but they do in many other places in the United States. Collecting better data would be a challenge because individual-level measurements are very expensive.

The next category of risk factors involves DDE, the metabolite of the pesticide DDT. Although the use of DDT has been banned in the United States since the 1970s, it is still used or approved for malaria control in 25 countries. The levels of the metabolites in humans are at now 5 percent of the levels found in those areas where DDT spraying is ongoing. In a recent study, Longnecker looked at the stored serum of U.S. women from the 1960s—a time when DDT

TABLE 4.1 Odds Ratio for Preterm Birth

DDE (µg/L)	Cases (n=361)	Controls (n=2019)	Odds Ratio for Preterm Birth*	95% Confidence Interval I
<15	34	375	1	
15–29	153	944	1.5	1.0–2.3
30–44	80	404	1.6	1.0–2.6
45–59	50	176	2.5	1.5–4.2
≥ 60	44	120	3.1	1.8–5.4

*A Preterm birth is defined as less than 37 weeks gestational age for this experiment.
NOTE: Trend $p < 0.0001$.

SOURCE: Modified from Table 2 in Longnecker et al., 2001. Reprinted with permission.

spraying was occurring. As shown in Table 4.1, there was an increased risk of preterm birth with increasing levels of DDE in serum (Longnecker, 2001). In the 2000s, most individuals have a very low (less than 15 micrograms per liter) DDE level. He noted that upon further examination there was a flat dose-response relation for exposures of less than 15 micrograms per liter. This suggests that DDE exposure as a risk factor for preterm birth may be more important in those countries where DDT is used for malaria control but not in the United States.

Longnecker reviewed a number of other potential risk factors for pre-term birth—many of which had few supportive data, conflicting results, or not enough information to make a conclusion. Chemical exposures such PCBs have shown an association in some studies, but further work is needed. Metals such as arsenic, cadmium, and lead have not been fully investigated. In Bangladesh, where arsenic levels are twice as high as in the United States, there is a suggestion from one study that there may be an association.

Alcohol Use

According to some workshop participants, a study done at the University of Washington suggests that women and their partners who used alcohol and drugs reported significantly higher levels of stress, weaker social support, and poorer levels of self-esteem (Lindenberg et al., 1999). Whether this accounts for an increase in preterm labor has yet to be investigated. The effects of alcohol on preterm birth are complex and can induce different pregnancy outcomes depending on the differential pattern and timing of exposure. In the rat and the rabbit, research has shown that parturition is prolonged if alcohol is given chronically (Cook and Randall, 1998). However, alcohol consumption on day 17 of gestation has been shown to induce preterm birth in the mouse. Kimmel reported that an ongoing study in humans suggests that moderate drinking tends to be associated with preterm labor, while social drinking has no effect.

Tobacco Use

Cigarette smoking by women during pregnancy continues to be a substantial contributor to poor perinatal outcomes in the United States. As discussed by many workshop participants, smoking during pregnancy is associated with low birth weight, premature birth, and reduced neonatal lung function. In addition to these effects on the fetus and infant, pregnant women who smoke are more likely to have problems such as miscarriage, premature rupture of membranes, and placenta *previa* (Surgeon General, 1989, 1994).

A clear dose-response relationship exists between the number of cigarettes smoked during pregnancy and the birth weight deficit. Compared with nonsmokers, light and heavy smokers have a 54 and 130 percent increase, respectively, in the prevalence of newborns weighing less than 2,500 grams (Surgeon General, 1989). The reduction in birth weight associated with maternal tobacco use seems to be a direct effect of smoking on fetal growth (Surgeon General, 1989). Mothers who smoke also have increased rates of premature delivery. The newborns are also smaller at every gestational age. The infants display generally symmetrical fetal growth retardation with deficits in measurements of crown-heel length, chest and head circumferences, and birth weight (Surgeon General, 1989). Low birth weight infants have a greater risk of neurological and developmental handicaps and congenital anomalies. In addition, they may be susceptible to respiratory infections as well as other disorders. Preterm low birth weight was associated with a higher risk of poor health and asthma among children when all the other selected risk factors were controlled. Poor maternal health and maternal smoking were important risk factors for poor child health (Chen and Millar, 1999).

Tobacco and Gene–Environment Interactions

Wang and colleagues investigated maternal cigarette smoking, metabolic gene polymorphism, and reduced birth weight in a U.S. population in order to examine whether maternal genotypes can modify the association between maternal cigarette smoking and infant birth weight. Two metabolic genes that are involved in benzene detoxification and excretion, CYP1A1 and GSTT, were used to assess a metabolic gene–smoking interaction. Consistent with previous data, the study found that maternal smoking during pregnancy was associated with reduced infant birth weight and gestational age. The study further demonstrated that the adverse effects of maternal smoking on infant birth weight and gestational age were modified by maternal genotypes, suggesting gene–smoking interactions.

CONVERGENCE OF CELLULAR PATHWAYS

The work of Rita Loch-Caruso, University of Michigan, provides insight into the cellular mechanisms by which some environmental chemicals such as DDT could initiate parturition. According to Loch-Caruso, it is often difficult to study toxicant effects that might influence parturition using the standard small laboratory animal models because of the diversity among species in initiating parturition. Her work is based on the knowledge that there is an apparent convergence of mechanisms for parturition at the level of the myometrium. At the level of the uterine smooth muscle, there are similarities across species in the myometrial responses that are necessary for parturition.

By recording contractions from the myometrium of pregnant rats in a standard organ bath, her laboratory has found that some toxicants, such as PCBs and DDT, can act directly on the myometrium to alter contractions. For example, DDT, which was described above as having a potential role in premature birth, was shown to increase the oscillations (contraction frequency) of the myometrium. The increased response persisted even after rinsing the strips with buffer (see Figure 4.2). In addition, PCBs, also candidates for reproductive effects, were tested in the organ bath. The commercial PCB mixture, Aroclor 1242, showed a dose-dependent effect on myometrial contraction. At 10 μM Aroclor, there was little effect; however 50 and 100 μM Aroclor increased the frequency of contractions.

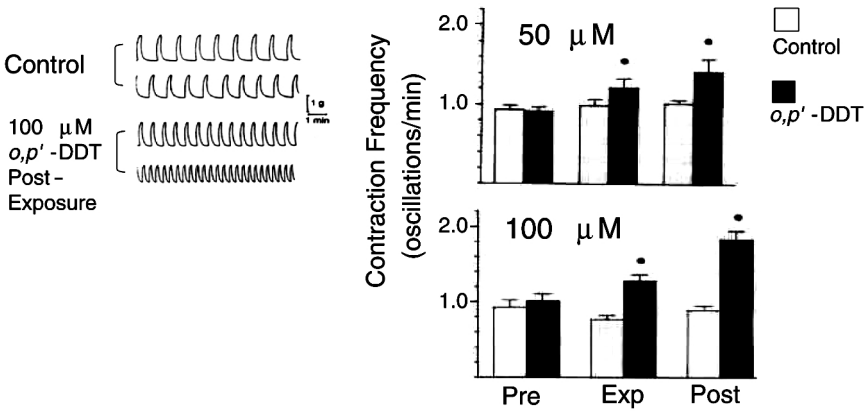

FIGURE 4.2 (a) Polygraph tracing showing the effect of 100 μM o,p′-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane) on rat uterine contraction frequency during the post-treatment period. The top two tracings are controls and bottom two represent DDT-treated strips, all of the same animal. The duration of the period shown is 10 minutes. (b) Effect of o,p′-DDT on isometric contraction frequency in rat uterine strips treated *in vitro*. SOURCE: Juberg et al., 1991. © 1991 Society of Toxicology. Reprinted with permission.

By understanding some of the basic cellular mechanisms of myometrial activation, Loch-Caruso and her colleagues have begun to improve our understanding of how chemical toxicants might recruit these normal processes. Aroclor 1242 (100 μM) was shown in her laboratory to increase the intracellular concentration of calcium by approximately 778 percent.

This increase was dependent on the influx of calcium through voltage-dependent calcium channels in the cellular membrane. Removal of calcium from the buffer (extracellular space), or blocking the L-type calcium channel with nifedipine, blocked the increase. The cellular mechanism by which this occurs is still under investigation; however, Loch-Caruso recently proposed that Aroclor 1242 increases phospholipase A_2 to release arachidonic acid, which works through a direct or indirect mechanism on the voltage-dependent calcium channels to increase intracellular calcium. There is some evidence for this pathway. Using reverse-phase high-performance liquid chromatography (HPLC) to analyze released arachidonic acid, 100 μM Aroclor 1242 increased the concentration of arachidonic acid (over controls) that was not converted to its metabolites. Further by using uterine strips in the organ bath, she was able to show that pretreatment with phospholipase A_2 inhibitors was able to block the stimulatory action of Aroclor 1242.

Loch-Caruso concluded that some environmental toxicants can act directly through cellular mechanisms to alter uterine contractions. In vitro approaches using laboratory animal myometrial tissue and cells may prove useful for assessing the potential risks of some environmental chemicals for pregnant women. She cautioned however, that these procedures will not detect chemicals that act through other mechanisms, for example, a toxicant that acts at the level of fetal and maternal signaling.

GENE–ENVIRONMENT INTERACTIONS AND PRETERM BIRTH

To date, most studies of preterm delivery have focused on social, environmental, or clinical variables. The role of genetic susceptibility and gene–environment interactions has been largely unexplored, in part because the study of complex gene–environment interactions involves dealing with a number of methodological challenges, including determining appropriate study design and the appropriate statistical analysis for testing the gene–environment interaction in relation to preterm births. Empirically, gene–environment interactions can be assessed in epidemiological model in which markers of genetic susceptibility can be incorporated to test these interactions. These regression models incorporate the genetic effect, environmental effect, and gene–environment interactions.

In studying gene–environment interactions, it is critical to accurately measure environmental exposures and to determine the genotypes. However, in reality, misclassification of environmental exposures can occur because of poor recall of previous exposures, complex patterns of long-term exposures, and lack of

good biological indicators of exposure levels. In addition, the timing of exposures, given the importance of the development of the fetus, is unknown, and there can be misclassification of genotypes. Study findings also could be biased because of confounding variables, from either environmental or genetic sources (e.g., linkage disequilibrium). Other unique challenges faced by those conducting research on preterm birth, compared to those conducting research involving other complex human diseases, are determining the role of maternal versus fetal genes in the metabolic pathways involved in the pathogenesis of preterm birth and identifying appropriate study designs and analytical methods for addressing this issue.

Inspired by previous work on the intergenerational influence on low birth weight, Xiaobin Wang of Boston University School of Medicine began to study the aggregation of low birth weight among Caucasians and African Americans in the United States by assessing the association between the birth weight of the mother and the index child and the risk of low birth weight among the siblings of that index child. The study consisted of a cohort of the 1988 U.S. National Maternal and Child Health Survey, and the analysis included 1,691 Caucasian mothers and 1,461 African-American mothers who had two or more live-born singleton children. The four study groups were defined as follows: (1) neither the mother nor the index child had a low birth weight; (2) only the mother had a low birth weight; (3) only the index child had a low birth weight; and (4) both the mother and the index child had low birth weights. As illustrated in Figure 4.3, the lowest odds ratio is found in the first group and the highest in the last. The pattern is similar for both Caucasian and African-American mothers. In fact, Wang et al. (1995) found that the risk of low birth weight and preterm birth was greatly increased if both the mother and the index child were of low birth weight. The findings persist in both African-American and Caucasian populations even after adjustment for maternal and infant characteristics. Wang suggested that the strong familial aggregation of low birth weight might be attributable to environmental factors, genetic factors, or both.

To begin to address the questions of gene–environment interaction, Wang began a second large-scale, epidemiological study of preterm births in a low-risk, homogeneous Chinese population as a function of benzene exposure. Wang's study sought to examine genetic differences in benzene metabolism and its relationship to the length of gestation. Benzene was of interest because it is a known reproductive toxicant and is a ubiquitous solvent. According to the National Institute of Occupational Safety and Health, an estimated 10 million U.S. workers are exposed to benzene. Further, most Americans are exposed to minor amounts of benzene through various sources, including the gastrointestinal system. Metabolism is essential for benzene detoxification, and it is speculated that an individual's reproductive risk associated with benzene exposure may be modified by genetic variation in metabolic detoxification activity. A two-phase pro-

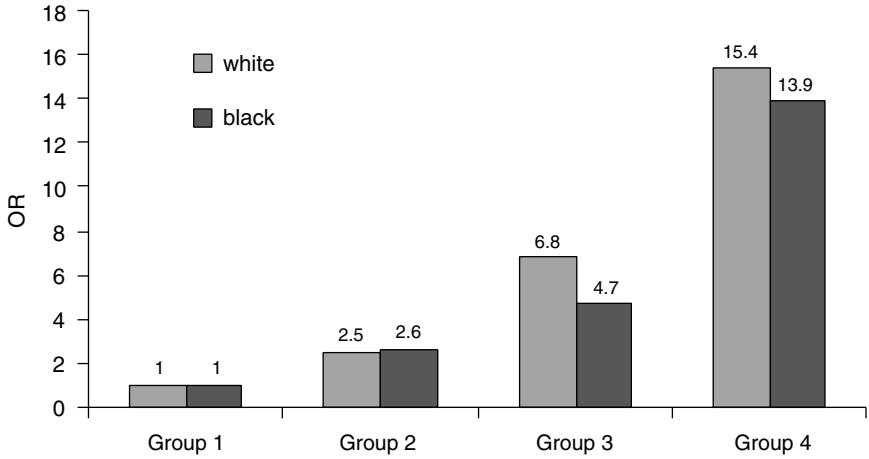

FIGURE 4.3 The risk of low birth weight may be predicted by whether the mother was low birth weight or whether she had a prior low birth weight infant (index child). The risk is lowest when neither the mother nor the sibling was low birth weight (group 1), only the mother had low birth weight (group 2), or only the index child had low birth weight (group 3). The highest risks were noted when both the mother and the index child were low birth weight (group 4).
SOURCE: Wang et al., 1995. © 1995 Massachusetts Medical Society. Reprinted with permission.

cess is necessary to detoxify and excrete benzene. The cytochrome P450 family, including CYP1A1, serves as the major enzyme system involved in phase one. Phase two relies on glutathione *S*-transferases such as GSTT1 to make the end product a stable hydrophilic compound that can be excreted easily.

The study population consisted of 542 postpartum Chinese female workers, of whom 302 had known low-level benzene exposure and 240 had no known exposure during pregnancy. As illustrated in Figure 4.4, benzene was shown to shorten the gestational age, which was dependent on maternal genotype. When the mother had the AA allele, there was a decrease in the gestational age at birth. The largest association occurred when the mother had AA allele and GSTT1 was absent. This study demonstrates that benzene exposure, even at a very low level, was associated with shortened gestation, thus suggesting a gene–environment interaction.

IMMUNE-DEFICIENT KNOCKOUT MICE AS A MODEL TO ELUCIDATE ENVIRONMENTAL TOXICANTS

Participants considered other animal models to begin to understand the effect of environmental toxicants on preterm birth and other reproductive abnor-

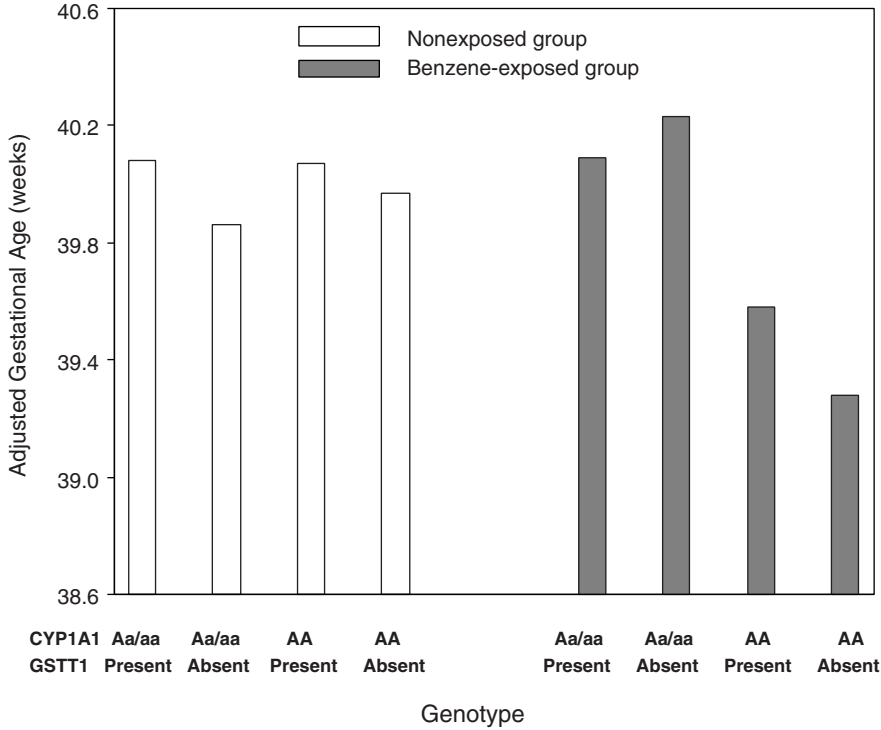

FIGURE 4.4 Gestational age by maternal benzene exposure status and CYP1A1 and GSTT1 genotypes, with adjustment for maternal age, education, parity, passive smoking, stress, prepregancy weight and height, and infant sex, Beijing, China, June 1995 to June 1997.
SOURCE: Wang et al., 2000. © 2000 Oxford University Press. Reprinted with permission.

malities. During her presentation, Anne Croy of the University of Guelph suggested that if mice lacking lymphocytes are housed under strict microbiological barriers, they are excellent models for reproductive studies because they are immune deficient.

The immune system is composed of highly mobile cells that normally circulate in the blood and have the ability to move in and out of tissues. Immune-competent cells move in and out of tissues by recognizing receptors on cells, particularly on endothelial cells, and by responding to gradients that can either attract or repel the lymphocytes within the tissue. The mammalian uterus is endowed with all of the known lineages of immune-competent cells and as such is a highly active immune organ.

In women, pregnancy is accompanied by the transient appearance in the uterus of a population of granulated lymphocytes. These specialized lymphocytes become the dominant immune cells in the first half of gestation and have been estimated to represent more than 70 percent of the maternal bone marrow-derived cells at implantation sites. This population, which has been defined in women and in mice as a subset within the natural killer (NK) lymphocyte lineage, has a strong association with uterine stroma. The granulated cells first appear and proliferate as uterine stromal cells transform from fibroblasts into decidual cells. Uterine natural killer (uNK) cells decline in the later part of pregnancy and are absent from the postpartum uterus.

Croy has found that mice that are genetically deficient in lymphocyte lineages have quantifiable implantation site defects. Specific genes are key to regulating and controlling the health of the site of uterine implantation. In one model, interferon-gamma (IFN-g), a key product of NK cells, appears to be a central regulator of the expression of these genes and could thus serve as a potential target molecule for monitoring the effects of environmental contaminants on the uterine immune system.

Establishing and quantifying consistent phenotypes in the uterine implantation sites of specific immune-deficient mice can provide powerful animal research tools in which reconstitution of implantation site structure can be assessed. These approaches involving reconstitution can be exploited in many ways in investigations of environmental toxicants, said Croy. She described a number of models (see "Models That Study the Action of Uterine Lymphocytes During Pregnancy" in the abstracts) that focus on uNK cells as indicators of environmental interference in the implantation process and, thus have some effect on the subset of preterm births that may be due to problems of implantation.

FEDERAL GOVERNMENT TOXICOLOGY PROGRAMS

In addition to the work at academic institutions, the federal government has a number of research and testing programs that are poised to test reproductive and development effects of chemicals. Three such programs are housed at the National Institutes of Health (NIH), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). The EPA has regulatory requirements for industry to conduct testing on pesticides and industrial chemicals, and the FDA has requirements for the testing of pharmaceuticals, food additives, and contaminants. The NIEHS National Toxicology Program conducts testing on a number of chemicals.

EPA's Risk Assessment Guidelines

In her role at the Environmental Protection Agency, Carole Kimmel has been actively involved in the development of a number of risk assessment guide-

lines, three of which are most relevant for a discussion of preterm birth and environmental chemicals: developmental toxicity, reproductive toxicity, and immunotoxicity. Current protocols use rodents as a model, although developmental toxicity testing also uses rabbits. Extrapolating the results from animals to humans may be problematic because of the differences in physiology. According to Kimmel, some of the differences are known, while others are not clearly understood. It is not clear from the literature how important some of these differences are for the human situation.

Developmental toxicity research looks at the adverse effects on the developing organism that result from exposure prior to conception (an exposure of either parent prior to the pregnancy), exposure during prenatal development; or exposure postnatally, to the time of sexual maturation. With the recognition that exposures during development may have effects that can be detected at any point in the future, there is a burgeoning area of research today on fetal origins of adult disease. The possible types of adverse developmental outcomes include death through early spontaneous abortions, perinatal death, or postnatal deaths; birth defects; altered or reduced growth; and functional deficits, e.g., mental retardation. Related to the work on developmental toxicity is the study of reproductive toxicity that includes adverse effects on the reproductive system, such as changes in gestation, parturition, lactation, onset of puberty, gamete production and transport, and reproductive senescence.

The EPA, along with other regulatory agencies, have developed a set of harmonized testing guidelines that are used by industrialized countries as guidance for testing of pesticides and industrial chemicals, according to Kimmel. These include the prenatal developmental toxicity study, which is typically done on rats or rabbits, although other species are used if warranted. Exposure occurs from day 6 of gestation, which correlates with the time of implantation in the rabbit, to approximately days 29–30 in rabbits or day 20 in rats—just prior to term, which allows for systematic evaluation of structural defects. Because of the testing procedure, it is not possible to study the issue of preterm delivery. From other research, it has been shown that rats shows prolongation of gestation rather a preterm delivery. Rabbits, however, do have preterm delivery, as early as days 17–18, but may also deliver as late as days 28–29. Thus, the rabbit may be a better model for understanding the effects of environmental exposures on preterm delivery.

Another study for which a harmonized testing guideline exists is the developmental neurotoxicity study, which was developed primarily to provide a better evaluation of developmental effects on the nervous system. It is used typically after a chemical has shown other indications of possible neurotoxic effects, in either adult or prenatal studies, and usually in rats. The original protocol was developed to start exposures around implantation and finish by postnatal day 10. However, the exposure period has recently been extended out to weaning (postnatal day 21), in order to cover even more of the developing nervous system. In

this type of study, it is possible to evaluate the timing of parturition and gestational length, and to measure both maternal and pup toxicity. The third testing protocol that is most relevant to the discussion of preterm birth is the two generation reproduction study, which is a standard study that is used for all pesticides. It is done mainly on rats, although the mouse may be used in some testing. In this protocol, the exposure begins before mating and continues until lactation in the F_1 generation. Selected offspring are continued with the exposure through mating and lactation of the F_2 offspring. This allows researchers to evaluate a long-term chronic exposure through two generations. The study is designed to look primarily at developmental effects as well as reproductive development and function. It follows development of the reproductive system over two generations, including gestation length. However, as indicated before, rats typically do not abort or deliver prematurely, even in the face of severe toxicity.

Drug Testing and Preterm Birth

The Food and Drug Administration has also developed testing guidelines and approaches for reproductive and developmental toxicity. These cover a number of drugs, chemicals, additives, and contaminants, and the testing guidelines are similar to those that EPA has developed for pesticides, according to Kimmel. For pharmaceutical agents, the approach to testing varies some, using what is called a three-segment approach. Segment I covers exposure from mating until the time of implantation. It follows the animals until they have offspring. Segment II is similar to the prenatal developmental toxicity study. The exposure is from the period of implantation to around the time of closure of the hard palate. Segment III is from the time of closure of the hard palate through weaning.

Animal Data on Preterm Birth

Through a review of the literature, Kimmel described some of the studies that have been reported on premature delivery from exposure. Using protocols similar to those in the testing guidelines, researchers found that parturition in the rat and rabbit is delayed if alcohol is given chronically. However, if alcohol is given acutely on approximately day 17 of gestation in the mouse, the animals experience preterm labor. According to Kimmel, there seems to be a differential effect of alcohol depending on the timing and pattern of exposure. A number of agents such as diethylenetriamine, tioconazole, and methyl methacrylate in rats, and prulifloxacin, idoxifene, and ostidine in rabbits, have been shown to cause premature delivery.

In concluding, Kimmel noted that the current testing guidelines do include some evaluation of gestation length, but the question remains whether researchers have the appropriate animal models to ask the appropriate questions regarding preterm birth. She suggested that current study designs may be able to be

modified to look at parturition in more detail. Currently, when effects are reported, it is generally at higher doses where there are other signs of maternal and developmental toxicity.

NIEHS's National Toxicology Program

The National Institute of Environmental Health Sciences' mission is to reduce the burden of human illness and dysfunction from environmental causes through a better understanding of environment, individual susceptibility and age, and how they interrelate. To meet part of this goal, the National Toxicology Program (NTP) was created to coordinate the toxicological testing programs within the Department of Health and Human Services and to strengthen science-based knowledge. This is an arduous task because there are approximately 80,000 chemicals approved for use in the United States, and approximately 2,000 additional chemicals are introduced annually.

A major emphasis of the NTP is to safeguard public health by identifying and characterizing toxic effects of environmental chemicals, including the effects on reproduction, said Jack Bishop of NIEHS. Research designs used by the NTP to assess reproductive toxicology include (1) the total reproductive capacity test (TRCT); (2) the dominant lethal test; (3) the reproductive assessment by continuous breeding (RACB) test; (4) the reproductive, development, and general toxicity (RDGT) test; and (5) the teratology test. He suggested that there may be opportunities for adjustment of these test methods to ask questions that are relevant to the issues of premature birth.

The total reproduction capacity test is primarily conducted in mice and generally involves single acute exposures. Approximately 30 females are treated per chemical dose group and concurrent control. After treatment, each female is caged with a male and mated for her reproductive life span. The end points measured are average litter size per mating interval, the proportion of females with young per mating interval, and the mean total number of offspring per female across all mating intervals. The TRCT was originally designed to identify environmental agents that might induce genetic damage in female germ cells or otherwise cause premature cessation of ovulation. The TRCT, as conducted for these purposes, does not capture length of gestation information needed to assess premature birth.

One of the classic toxicology study paradigms is the dominant lethal assay, according to Bishop. Male or female animals are given either a single or multiple exposures to a chemical. The females are checked for the presence of a vaginal copulatory plug, so the time of mating is known in this test. With this paradigm researchers measure the number of implantation sites, the number of viable and dead fetuses and the number of resorptions. A minimum of 300 implantations per dose group need to be analyzed for an adequate test. The conduct of a female dominant lethal test is more complex than a male dominant lethal test because

true genetic damage to the oocyte can be easily confounded by maternal toxicities. The disadvantage of this paradigm for the study of preterm birth is that the females are sacrificed prior to term, which means that information regarding gestational length cannot be obtained.

The third paradigm is reproductive assessment by continuous breeding. Exposure occurs during the F_0 and F_1 generations from prior to conception through weaning. Similar to the EPA two-generation study, some F1 offspring are exposed and mated. Generally during this experiment they do not check for vaginal copulatory plug, so data on gestational length is not captured. "Average Gestation Length" has been determined in select studies where plug checks have been performed and another statistic, "Cumulative Days to Litter", has been calculated in the absence of plug check data (see Table 4.2).

The final assay is the reproductive, developmental, and general toxicity protocol. This assay or study paradigm has many versions that can look at different outcomes (gestational length, average time to litter, cumulative time per litter, numbers of pups per litter, decreased pup weight, altered pup sex ratio, etc.), and may be applicable for studying preterm birth.

Of the chemicals reviewed to date using either the RACB or RDGT paradigms, only three exhibited a significant exposure-related change in gestation length (see Table 4.2): ethoxyquin increased the time to litter, and sodium selenate increased the gestation length. Only nitrofurazone decreases the average time to litter.

The current animal models that test for chemical toxicity may be inadequately designed to capture information on chemically induced alteration of gestation length.

Jack Bishop

The current animal models that test for chemical toxicity may be inadequately designed to capture information on chemically induced alteration of gestation length, according to Bishop. We don't report many effects, which may suggest that we need to look at the experimental design. Further, the rodent model has a number of limitations. The hormonal milieu of the rat and mouse are very different from that of humans. Another critical issue, in terms of gestational length, is that in the rodent we must measure "hours of gestation," which current protocols are not designed to measure. He further suggested that we need to consider developing alternative models to better test for potential environmental effects on preterm birth.

TABLE 4.2 Chemicals Reviewed in RACB 1/N RDGT Studies

Average Gestation Length[ST]	Cumulative/Average Days to Litter
Bromoacetonitrile	Methylene Blue trihydrate[T2/4]
Bromochloroacetic acid	***Nitrofurazone*** ↓
Bromodichloromethane	Propylthiouracil [F0/1]
Chlorodibromomethane	Sodium Bromate[F0/1]
Dibromoacetonitrile	Tetrachlorobenzene[F0/1]
Hexachloroacetone	Thiophenol[T2/4]
Sodium bromate	AZT[F0 F0/1]
Sodium selenate ↑	CD1
(22 vs 23 days)*	BTCA[F0/1]
Tribromoacetic Acid	Caffeine[T2/4]
Sodium Bromate	Dibutylpthalate[F0/1]
Elmiron[T2/4]	Dicyclopentiadine[T2/4]
Methacrylonitrile[T2/4]	Diethylhexylpthalate[F0/1]
Tamoxifen citrate[F1]	**Ethoxyquin**[T2/4] ↑
Potassium dichromate[T2/4] BalbC 19.5	Hexachlorobenzend[F0/1]
	Indium trichloride
	Tetrahydrate[T2/4]
	Isoeugenol[F0/1]

Of the 30 RACB-RDGT studies reviewed, only three chemicals altered either time to litter or gestational length. The remaining chemicals listed in this table have been tested but not found to have an effect on either time to litter or gestational length. Ethoxyquin increased the time to litter and sodium selenate increased the gestational length. Only nitrofurazone decreased the average time to litter.
* Sodium selenate increased the time of gestation from 22 to 23 days.

SOURCE: NIEHS/NTP Reproductive Toxicology Database. Reprinted with permission.

5

Preterm Birth—Social Implications*

As discussed in Chapter 4, a recurrent theme in the Roundtable discussions has been the role of an expanded definition of environment, including the social environment, and its impact on human health. During the workshop, participants discussed the evidence suggesting an impact of the social environment on pregnancy outcome. Janet Rich-Edwards, Harvard Medical School, suggested that whether evaluating a social or environmental toxicant, it is important to look at the timing and duration of the exposure. In addition, she suggested, it is worth investigating factors that might have been present before the pregnancy was initiated to try to identify social predictors of preterm birth, to explore the concept of women becoming weathered or worn down by stress, to assess whether these social predictors endure over time, and to differentiate between the effects of chronic and acute stress.

Whether evaluating a social or environmental toxicant, it is important to look at the timing and duration of the exposure.

Janet Rich-Edwards

* This chapter and subsequent chapters were prepared from the transcript of the meeting by a rapporteur. The discussions were edited and organized around major themes to provide a more readable summary and to eliminate duplication of topics.

STRESS

As more researchers and clinicians recognize the impact of stress resulting from an unhealthy environment on human health, stress has become the subject of intense research in the last decade. Previous research has established that low levels of stress activate cellular pathways that are necessary for growth, development, and maintenance of brain and muscle activity, as well as learning and memory. However, prolonged exposure to a chronic stressor or exposure to an acute stressor that overwhelms the individual's ability to return to homeostasis can result in ill health. The individual's ability to handle stress depends not only on the strength of the agent but also on the host's susceptibility to stress and on the social environment that surrounds the individual. Social environment factors such as education, family status, household income, race and ethnicity, social support, coping, and repressive coping style, as well as violence, can predetermine the outcome of pregnancy. The individual's degree of social stress is related to the stability of the pre- and postnatal social environment in which it lives, the amount of social support which the individual receives from its peers and family, and its social experiences during behavioral development.

Two kinds of stressors have been most extensively studied with respect to their impact on pregnancy outcomes: acute stressful life events and hard physical work—a type of individual chronic exposure. A third kind of stressor, individual experiences of racism, according to some participants, may explain at least part of the excess preterm delivery rate among African and Mexican Americans who have lived in the United States for most or all of their lives. However, said Fernando Guerra, San Antonio Metropolitan Health District, some of the conditions revealed in the data about the health of Mexican Americans, related to nutrition, diet, life-style, risk-taking behavior, domestic violence, displacement from the security of family and home, conditions of overcrowding, and lack of attachment to strong religious ties, indicate that these are stressors that must be considered and studied further.

Epidemiological Model of Stress

Carol Rowland Hogue of Emory University suggested that the classic "host, environment, agent" triangle of epidemiological causality can illustrate what might be included in a more comprehensive test of the stress–preterm delivery hypothesis. In this model, the host is the individual woman, the environment is her social and cultural context, and the agent is the immediate stressful event(s) requiring her response. This framework provides a context to determine the extent to which a given theory may include important and potentially interrelated factors.

In considering host susceptibility, it is important to note that individuals may differ in psychological and physiological responses to the same stressor,

reflecting differences in the context within which the stressor occurs and differences in individual response to that type of stressor.

Research Application of the Model

Three studies have found that when pregnant women express anxiety about their pregnancy, they are more likely to experience a preterm delivery. These studies collected information on pregnancy anxiety prior to delivery; however, it is not possible to ascertain whether subtle pregnancy complications, which might have alerted the women to impending problems, had triggered the pregnancy anxiety and, therefore, were also the cause of the preterm delivery, according to Hogue.

In one small study among pregnant women, elevated blood pressure—in response to an arithmetic test—was associated with both decreased birth weight and gestational age. In experimental studies of African-American and Caucasian participants, blood pressure following cardiovascular activity, at rest, and after a stressful stimulus was greater in African Americans. This increased cardiovascular reactivity among African Americans does not appear to be associated with familial history of hypertension, suggesting that individuals' exposure history, rather than genetic differences, may explain differences in host susceptibility to stressors. Early and continuous stressors may also cause learned physiological responses that trigger higher reactivity when similar stress occurs later in life. For example, early and continual experiences of individual racism have been hypothesized to sensitize African-American women to stress reactivity, said Hogue.

Previous and Acute Stress in the Household

The issue of women being weathered or worn down by stress, also known as weathering, suggests that the environment is literally incorporated into the host. Environment may weather a woman and increase her susceptibility to giving birth to a premature baby through the altered hormonal responses to stress, according to Rich-Edwards. For example, repeated exposure to threats during childhood, such as may be encountered in a violent household, may repeatedly activate the hypothalamic–pituitary–adrenal (HPA) axis response to stress and actually reshape that axis. Entering adulthood with an altered HPA axis, a female may find herself with a hair-trigger response to stress, or "kindling" or "arousal pathology."

Violence and chronic stress in the household can potentially have profound implications on reproductive health, according to Rich-Edwards. One study in Glasgow, Scotland, showed that chronic strain in the household was associated with both preterm delivery and low birth weight. Further, data showed that mean

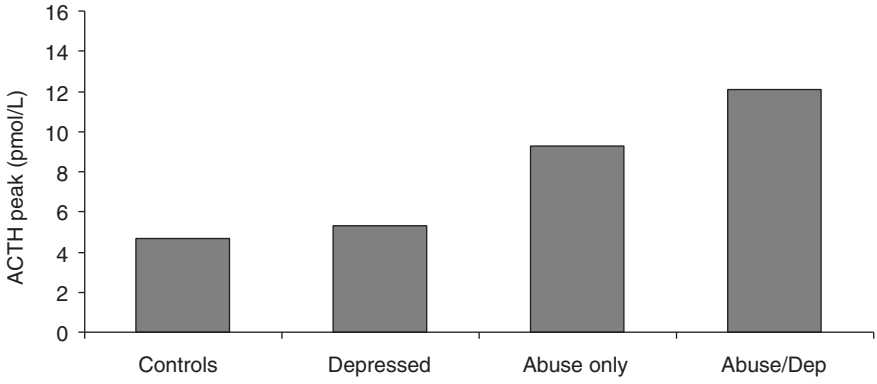

FIGURE 5.1 Mean peak ACTH responses to a laboratory stressor were significantly elevated in women who were abused as children compared to controls or those who were clinically depressed. Further elevated responses were noted in those women who were abused and also showed signs of clinical depression.
SOURCE: Heim et al., 2000. © 2000 American Medical Association. Reprinted with permission.

peak Adrenocorticotropic hormone (ACTH) response to laboratory stressors is higher among women who were abused as children than among those who were not (Figure. 5.1).

Early abuse was associated with an exaggerated ACTH response, whether or not the women experienced depression afterwards. Differences were also observed in cortisol response and heart rate response among women who were both abused and depressed.

Rich-Edwards believes that the grim statistics surrounding violence may potentially have profound implications for stress response among women. A nationally representative phone survey of 8,000 women ages 18 or older conducted from 1995 to 1996 by the National Institute of Justice and the Centers for Disease Control and Prevention asked women about their lifetime exposures to violence. The survey found that one out of every two women had been physically assaulted at some point in her life, either as a child by an adult caretaker or as an adult (Tjaden et al., 2000). In addition, one out of every six women experienced rape or attempted rape. Data on the rate of intimate partner violence to women showed a marked association between low income and rate of abuse.

Of those who experienced rape, 22 percent were raped before age 12 and another 32 percent were raped between the ages of 12 and 17, indicating that the majority of these victims were raped as children. According to some participants, we need to understand further the role of the various types and levels of stress on premature birth.

SOCIAL SUPPORT

Another consideration is the environment or the social and cultural context within which a woman lives. Ameliorating environmental factors include intimate social support, which has been associated with improved pregnancy outcomes in a number of studies. However, attempts to replicate social support in clinical trials to prevent poor pregnancy outcomes have not been effective. The chronic stress associated with environmental or social stressors may be measured at the individual level, for example, through inquiring about the level of daily difficulties experienced as well as about perceptions of environmental issues. However, the negative health impact of environmental stressors depends not only on individual perception, but also on actual environmental risks. For this reason, measurement of environmental risks must include contextual variables. Types of stressful environmental factors include those associated with gender, socioeconomic status, and race or ethnicity.

Several years ago, a series of studies found that maternal stress during pregnancy was associated with poor pregnancy outcomes, including preterm delivery, but only for women who had fewer social supports available to buffer stressful events. Subsequent studies of stress, social support, or combinations of stress and social support conducted in many settings have produced only a few significant findings and usually with small odds ratios. Interventions aimed at increasing social support have, in general, not lengthened gestation, said Hogue. The lack of success in these trials could be due to problems in design or analysis, or the trials may have been diluted by including women in the intervention group who did not need social support. If the presence of stressful events combined with the lack of social support during pregnancy does increase the risk of preterm delivery, then randomized trials that increase social support for women in need of such support should serve to lengthen gestation.

> Interventions aimed at increasing social support have, in general, not lengthened gestation.
>
> *Carol Rowland Hogue*

SOCIAL POSITION

The association between poverty and poor pregnancy outcomes has been found consistently across populations over time and by various measures of social class and social status. Causal models explaining this association point to the greater exposure to negative life events experienced by poor individuals. Additionally these individuals have fewer coping resources available for adapting to stress, and traditionally, they live within a culture of poverty that encourages hopelessness and resorting to unhealthy coping strategies. Also, poor communities are more exposed to environmental hazards such as lead, toxic agents,

and unsafe neighborhoods, and they do not have adequate community resources for responding to health and medical emergencies. Including these community-level factors in studies of stress and preterm delivery requires group-level measurements of exposure. Such factors have not been utilized widely in epidemiological research in these areas, but a few studies are beginning to confirm the utility of pursuing these contextual causes.

Social position, Rich-Edwards noted, is an example of a strong predictor of preterm birth, which has implications for the timing of chronic stress. Women's education level is a predictor of low birth weight births across racial and age lines. In all categories, however, African-American women are more likely to have low birth weight babies than Caucasian women. For example, a college-educated African-American mother still has twice the risk of having a low birth weight baby compared to a Caucasian college-educated mother. Further, among African American infants, mortality is lower in cities with less residential segregation (an example of institutional racism), a finding that is independent of the effect of poverty on infant mortality. In addition to the growing evidence that institutionalized and interpersonal racism adversely affects the health of African-American infants, racism in the United States may also affect the reproductive health of Mexican Americans.

> Women's education level is a predictor of low birth weight births across racial and age lines.
>
> *Janet Rich-Edwards*

COPING STRATEGIES

Coping strategies, in contrast to coping resources, describe specific behavioral or cognitive attempts to manage stressful demands. Problem-focused coping is more likely to be used when the stressor is perceived as controllable or modifiable, while emotion-focused coping is used more frequently when the stressor is perceived as uncontrollable. To date, research results are inconclusive regarding whether either strategy is more effective in buffering the effects of stressors, according to Hogue. Another important buffer may be spirituality, or reliance on a force beyond the individual. This too has yet to be explored with respect to preterm delivery. Among the most common coping strategies known to have deleterious effects on the outcome of pregnancy are alcohol use, drug dependence, and cigarette smoking.

6

Future Directions*

OVERVIEW

Preterm birth is a complex, multifactorial public health issue that is a growing problem in the United States and Canada. It continues to be a significant public health burden that has received little attention outside the prematurity research community. In the future, we need to think more critically about the impact on health if we are going to improve our performance in meeting the challenges posed by preterm births, asserted Donald Mattison.

The roots of preterm birth may start in early pregnancy, while some believe that the path to preterm birth starts before pregnancy, and may go back as far as the parent's early life.

Mark Klebanoff

Many of the participants agreed that preterm birth is not an acute event. The roots of preterm birth may start in early pregnancy, while some believe that the path to preterm birth starts before pregnancy and may go back as far as the parent's early life, according to Mark Klebanoff, National Institute of Child Health and Human Development. Similarly, researchers are recognizing that preterm birth is a "catch-all" phrase for the multiple pathways leading to a clinical presentation. At the fundamental level, Allen Wilcox, National Institute of Environmental Health Sciences, suggested that we may be looking at two different types of prematurity. The first would be a fetus that is in trouble from the early stages of pregnancy (e.g., problems of placental implantation, a fetus in distress).

* This chapter was prepared from the transcript of the meeting by a rapporteur. The discussions were edited and organized around major themes to provide a more readable summary and to eliminate duplication of topics.

The second situation would be a perfectly healthy fetus that for some reason is subjected to the natural events of delivery too soon. A number of presenters speculated that perhaps not all preterm birth is bad, and in fact, it may serve as a protective mechanism in some cases for the fetus or the mother. Yet, overall, the reason for the health burden from preterm birth is summarized as increased mortality and morbidity.

RESEARCH OPPORTUNITIES

This workshop represented a unique opportunity for the Roundtable and participants to look at the field of premature birth and the research opportunities that intersect with environmental health. The insight provided by bringing together investigators from diverse disciplines, including social and behavioral sciences, toxicology, and reproductive biology, clearly points out the heterogeneous and complex nature of the factors associated with preterm birth. While planning for future research, some participants suggested that we need to look at the complexity of the process and that preterm birth will have to be treated as a chronic disease—one might see this as being similar to our approach to studying hypertension, cardiovascular disease, or diabetes.

Many speakers alluded to the idea that we are on the verge of understanding the factors underlying preterm birth at the molecular, individual, and community levels. Armed with this knowledge, we will be in a better position to develop intervention strategies to address many types of preterm birth (see Box 6.1). However, the real questions that need to be asked, according to one panelist, are how are we going to get there and how are we going to get there with great dispatch? We must bring together biological, psychological, and social research to form a partnership for science—similar to the 1960s National Aeronautics and Space Administration (NASA) project to put a man on the moon. This would require a commitment of resources to make a significant impact. Box 6.1 is a compilation of the strategies identified during these discussions.

Improving National Surveillance and Registration

Terminology

Many speakers pointed out the variety of ways to classify premature delivery. They suggested that the criteria for defining term and preterm labor should be reassessed because they can alter the interpretation of the data as well as of the risks. For example, the current data are blurred by a lack of distinction between indicated preterm labor and spontaneous preterm labor. Determinations of gestation length could be improved by the use of an additional measurement beyond calculations based on the last menstrual period. Several speakers questioned whether the appropriate measure might be preterm birth or gestational

BOX 6.1
Major New Research Opportunities

During the workshop, participants listed a number of research opportunities for research in preterm birth. These included the following:

1. Improved animal models for the various etiologies of premature birth.
2. Molecular classification of preterm stages by genomics-based assays.
3. Molecular classification of exposures by improved genomic-based assays.
4. Further definition of biological pathways involved in term and preterm birth.
5. Improved surveillances and registries

length, and whether these variables should be discussed as dichotomous or continuous variables.

Data Collection

Because it is expensive and time-consuming to create new birth and newborn registries, Karla Damus suggested that efforts should be made to improve already existing registries. We need to review the content and types of information we are collecting to make them more relevant to issues of prematurity by adding well-thought-out case definitions to selected maternal and child health outcome registries.

Consequences of Prematurity

During the opening remarks and the closing discussions, participants suggested that we do not have a full understanding of the consequences of prematurity. They noted that current attempts have looked at short-term outcomes and that the full spectrum of the impact of preterm birth over the life of the individuals has not been fully studied with respect to disabilities of all types, including neurodevelopmental disabilities. For this reason, some individuals suggested that it would be important to describe more completely the impact of preterm delivery on the life course, including individual, family, and social impact.

Mechanisms of Preterm Birth

Some participants expressed amazement that they could not describe the initiation of labor and delivery in the normal pregnancy and suggested that this continues to impede our ability to understand mechanisms underlying preterm

birth. Without basic understanding of normal labor and delivery processes, we will not be able to study premature birth systematically. Others, however, believed that we are very close to describing the basic mechanisms and that we will be able to make significant progress by the end of the decade. Charles Lockwood discussed the need to understand gene–environment interactions (see below). The current risk factors do not accurately predict which women will deliver early. For example, maternal stress, infection, decidual bleeding (abruption), and uterine abnormalities have been implicated in preterm birth. However, the majority of women with these risk factors do not deliver prematurely.

In his closing summation, Mattison pointed to a number of the risk factors as a starting point for addressing preterm birth. He suggested that researchers should explore why prior preterm birth is one of the strongest risk factors for subsequent preterm delivery. Further, researchers may have to understand how several or multiple risk factors interact to increase the risk of preterm delivery. Better definitions and fully characterizing the interactions between birth weight and gestational age, the interactions of genetic factors and the environment, and differential mechanisms of preterm birth as a function of gestational age provide some direction to tease apart the complexity alluded to during the course of the workshop.

Genetics, Environment, and Gene–Environment Interactions

The study of genetics, environmental agents (including chemical, physical, and biological exposures), and gene–environmental interactions in relation to preterm birth is at a very early stage. Recent advances in molecular biology and the sequencing of the human genome will help to guide the field further. Researchers pointed to the fact that there are three genomes and environments involved—those of the mother, the father, and the fetus. Understanding these genetic roles and their interactions with the environment will be important for making the next leap in addressing issues of prematurity, concluded Mattison. To begin to address these issues, participants discussed the intersections between the environment and the genome.

Environmental Impacts

Participants and panelists discussed a variety of environmental factors that may contribute to the problem of premature birth. People had listed a number of toxicants and/or classes of toxicants that warranted further study. Endocrine disruptors, such as 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) and its metabolites, were discussed as one area of interest for the field of preterm birth. Participants suggested that further study was needed to understand the impact on preterm birth. Of special concern would be the endocrine disruptors that fall into the estrogen agonist/antagonist categories because of the delicate balance of hor-

mones during pregnancy. Some participants wondered if a shift in the balance between estrogen and progesterone could explain some preterm birth.

During the course of the workshop, participants spoke of the environmental effect on the myometrium, but they also said that we know little about the effects of exposures on the cervical collagen or rupture of the amnion. Understanding how these environmental factors influence the cellular and molecular pathways will be of continued importance in the next few years.

Social–Behavioral Factors

On the first day of the workshop, participants discussed the growing influence of those social environmental factors that influence preterm birth. Participants questioned of the impact of the environment and life-style factors on all aspects of implantation, fetal timing, and the following initiation of parturition. Some areas that might help to shape the field include the following:

- reframing research questions about the etiology and mechanisms of preterm delivery to take advantage of new knowledge about community, family, individual, social, economic, and environmental factors on the organism, as well as cellular, molecular, and genetic mechanisms of preterm delivery,
- understanding the impact of social factors on the biological mechanism of preterm delivery,
- understanding the impact of stress on maternal physiology on premature delivery,
- encouraging cross-disciplinary approaches that would allow more critical evaluation of the role of stress and of the biological mechanisms produced by stress as they influence preterm delivery, and
- exploring the role of stress on androgen and estrogen levels in pregnancy and their influence on gestational length, especially given the growing knowledge concerning environmental endocrine disruptors.

Gene–Environment Interactions

Participants discussed the tremendous opportunities for future research. As this research avenue moves forward, gene–environment interactions in this area must be combined with rigorous evaluation of reproductive health end points and potential confounding factors. This means that researchers have to know more about biological markers and their significance. Two areas that were highlighted included exploring (1) the interactions of fetal and maternal genetic factors contributing to preterm delivery, and (2) the interactions of environmental exposures such as tobacco and genetic factors. In his summation, Mattison recognized the importance of these areas and suggested that it will be necessary to fund research to explore both individual factors, and their interactions.

Susceptible Populations

The workshop highlighted the tremendous complexity of prematurity that needs to be addressed. Participants noted that there is tremendous variability between nations and ethnic groups, even among developed countries. For example, Caucasians in the United States have approximately twice the incident rate of Caucasions in France for reasons that are not obviously apparent. To begin to understand the population differences both here and abroad will require additional research.

Fernando Guerra suggested that we must address changing demographics. There is still a considerable amount of research that has to be done that will enhance the discussion of relevance to public health, including at the community level. He further suggested that we must look at special populations such as recent immigrants to the United States. Many of the participants echoed a need for a surveillance system that will allow us to follow various populations over time. Mark Klebanoff suggested that we should not get too focused on biology. Any biological mechanism must be reconciled with preterm births being a disease of poverty. Spontaneous preterm birth occurs in almost every place that has been looked at, but it is more common in cases of social adversity.

Animal Models

To begin to address some of the research questions, we will also have to develop good animal models for prematurity, according to Carole Kimmel. It is difficult to cause prematurity in the rat, which suggests that we must look at other animals, such as the rabbit, to better understand the process. Further, others suggested that we will need to develop animal models for each of etiologies underlying prematurity. Developing these models will help screen for potential environmental interactions and establish priorities for toxicological evaluations. In addition, this research will provide data about receptor interactions that will help guide future research.

Molecular and Biological Considerations

Molecular classifications of exposure have much to offer since true prospective studies that begin prior to or early in pregnancy allow for assessment during the etiologically relevant time interval, according to some panelists. Biological markers of nutritional status (e.g., serum folate, ferritin, transferrin receptor saturation), stress (e.g., corticotropin releasing hormone, cortisol), tobacco use (e.g., urinary and serum cotinine), cocaine use (e.g., cocaine and benzoylecognine in hair and urine), and infection or inflammation (e.g., fetal fibronectin, cytokines) have all been incorporated into epidemiological studies of preterm birth to great benefit. In particular, participation in prenatal care offers unusually favorable

opportunities to incorporate the collection of biospecimens in large clinically based populations, as opposed to community samples. The collection of such biospecimens has clear applicability to the study of environmental agents because biospecimens are key in determining the toxic mechanisms of suspected agents as well as for measuring exposures.

Risk Assessment

Exposure studies have methodological limitations that must be addressed before useful interpretations can be made about how exposure affects preterm labor, noted participants. Some participants suggested that we must increase our basic knowledge of risk assessment and toxicology as they relate to preterm birth, including exposure (e.g., quantity, timing, and dosage) and outcome measurements (e.g., gestational age, initiation of labor, birth outcome, status of the child into early childhood). In addition, improved study designs and approaches are needed to better measure exposures in longitudinal studies. For example, it would be useful to know the range or types of exposures that are significant for fetal implantation problems or the range of possible exposures necessary to initiate maternal complications. We also need to know how these complications overlap and affect one another.

Interventions

Strategies developed to address prematurity have focused in the past on means of reducing mortality; however, as Maureen Hack discussed, we may be at the limits of viability with present neonatal technology. Further, these strategies have failed to make a significant impact on morbidity, which suggests that researchers and clinicians must focus more clearly on other data and issues to address problems of morbidity.

Many panelists reminded participants that the goal of any pregnancy was to have healthy mothers and healthy babies. They suggested that we would have to determine those incidents in which when medically indicated, it would be better to have the baby born premature than to prolong the pregnancy. However, for cases in which it would be advantageous to prolong the pregnancy, some speakers felt that we were close to understanding some of the pathways leading to prematurity and, thus, close to developing intervention strategies. Mattison suggested that by the end of the decade we should have effective intervention strategies for many of the potential pathways. He further suggested that we need to be able to think strategically about intervening. This was echoed by another participant, who further asserted that the basic science has to be integrated with applied science. Too often during the workshop, speakers and participants discussed intervention strategies that had been implemented but had no benefit, or even had ill effects. A number of speakers asserted that we must study interven-

tions in randomized control trials before we implement such strategies on a population base.

Methodological Challenges

Influences on preterm birth have been more difficult to identify than causes of reduced birth weight. The predictors of reduced birth weight are sometimes shared with predictors of preterm birth, but often have weaker associations. Some predictors of preterm birth, such as socioeconomic status and tobacco use, seem to have strong influences on birth weight, but only modest influences on preterm birth, indicating that the pathways, although unidentified, probably overlap to some extent. Direct examination of preterm birth, low birth weight, and small-for-gestational-age deliveries suggests that modest overlap occurs.

One reason for the more limited success in the study of preterm birth may be the markedly greater uncertainty involved in the measurement of duration of gestation as compared to birth weight, reflected in the greater magnitude of error in vital records, the lower quality of maternal reports of gestational age compared to birth weight, and the more limited availability of gestational age from less developed countries. Duration of gestation is usually based on the last menstrual period—a fallible marker of the time of ovulation—although the widespread use of ultrasound for dating has been helpful in increasing accuracy in many settings and has been of benefit to those studies that can incorporate this information.

Isolating specific causal agents from nonspecific influences of a healthy life-style and favorable socioeconomic conditions will be very difficult, cautioned David Savitz. For example, consistent findings have emerged that identify an association between cocaine use and preterm birth, a reduced risk associated with leisure time physical activity during pregnancy, a reduced risk associated with favorable nutritional status and the use of prenatal vitamins, and an increased risk associated with physically demanding occupations. However, none of these associations is necessarily causal, and distinguishing a true etiologic effect from a spurious association due to other unmeasured or unknown factors has been unsuccessful thus far. Isolating environmental agents from the circumstances that give rise to exposure will pose a serious challenge for the identification of the many environmental agents associated with socioeconomic deprivation and less favorable life-styles.

Abstracts

EVALUATING CHEMICAL AGENTS FOR POTENTIAL HAZARDS TO REPRODUCTION

Jack B. Bishop

The mission of the National Institute of Environmental Health Sciences (NIEHS) is to reduce the burden of human illness and dysfunction due to environmental causes through a better understanding of interrelationships between the interactive elements of environment, genetics (susceptibility) and time (age). The National Toxicology Program (NTP) at NIEHS strives to safeguard public health by identifying and characterizing the toxic effects of environmental chemicals and by providing quality data that regulatory agencies can use for risk assessments.

NTP tests that assess reproductive toxicity include (1) Total Reproductive Capacity Test (TRCT; Bishop et al., 1997); (2) Dominant Lethal Test (DLT; Generoso and Piegorsch, 1993; Lockhart et al., 1992); (3) Reproductive Assessment by Continuous Breeding (RACB; Chapin et al., 1997;1998); (4) Reproductive, Development, and General Toxicity (RDGT); and (5) Teratology Test (TER). These are primarily rodent tests conducted with rats and/or mice, except for the TER, which may also include rabbits, hamsters, or guinea pigs. Taken together, these tests cover periods of exposure from the gametes through, fertilization, implantation, in utero development, birth, lactation, postnatal development, and the adult, even out to a total of three generations.

Most of these tests are conducted at NTP contract laboratories under carefully specified protocols. However, these test protocols permit variations in the sex, species, and strain of test animal, route and dosing regimens for chemical administrations, and even the number and types of endpoints measured. A primary endpoint in all of these tests is pup survival, either number in utero or

number of live born. Other endpoints evaluated may include body weight, feed consumption, clinical signs, pup or fetal weights, anogenital distance, neurological functions, immunology, hormone analysis, sperm parameters, vaginal cytology, organ weights, gross and microscopic pathology, and visceral and skeletal anomalies. Gestation length can be determined with most of these assays, but often the matings are not specifically timed.

The NTP has conducted female mouse TRCTs on 55 chemicals, male mouse DLTs on 30 chemicals, female mouse DLTs on 30 chemicals, mouse RACBs on 80 chemicals, rat RACBs on 36 chemicals, rat RDGTs on 12 chemicals, mouse TERs on 46 chemicals, rat TERs on 51 chemicals, rabbit TERs on 23 chemicals, and hamster TERs on 2 chemicals. None of the TRCT, DLT, or TER studies identified any significant change in gestation length, although there were numerous studies with significant reductions in pup survival and/or reduced size and weight. Of 20 RACB and RDGT studies reviewed to date for which either "average gestation length" or "cumulative time to litter" data were recorded, only 3 exhibited a significant exposure-related change: one reduced the average time to litter (nitrofurazone), one increased time to litter (ethoxyquin), and one increased gestation length (sodium selenate).

Currently, plug checking and/or vaginal smears are conducted for DLTs, RDGTs, TERs and the RCAB F_1 generation matings such that gestation length can actually be determined, but assessment of "prematurity" is difficult. It may be necessary to modify some of the current NTP reproductive test protocols to better collect this information. However, considering the infrequent observation of a significant effect, especially a shortening of gestation that might be associated with "preterm delivery, one has to question whether it would be worth the associated increased test cost.

Further, the role of infection in the etiology of premature birth has been estimated to be 40–60 percent; it is doubtful that rodent models will be of much use in predicting such environmental effects. In addition, initiation of parturition, a critical element in preterm deliveries, appears to be vastly different for rodents than for primates (Norwitz et al., 1999). Thus, I think we must constantly reassess our use of rodent animal models and the endpoints we measure for predictors of human ill-health effects, particularly for outcomes such as preterm birth.

MODELS THAT STUDY THE ACTIONS OF UTERINE LYMPHOCYTES DURING PREGNANCY

B.A. Croy

The mammalian uterus is endowed with all of the known lineages of immune-competent cells. In adult females, predictable, cyclic patterns of change occur in immune-competent uterine cells that can be correlated with ovulation or

pregnancy. In women, many nonhuman primates, and rodents, early pregnancy is accompanied by the transient appearance in the uterus of a population of granulated lymphocytes. These specialized lymphocytes become the dominant immune cells in the first half of gestation and have been estimated to represent >70 percent of the maternal bone marrow-derived cells at implantation sites. This population, which has been defined in women and mice as a subset within the natural killer (NK) lymphocyte lineage, has a strong association with uterine stroma. The granulated cells first appear and proliferate as uterine stromal cells transform from fibroblasts into decidual cells. Uterine Natural Killer (uNK) cells decline in the later part of pregnancy and are absent from the postpartum uterus.

Studies of pregnancies in mice genetically depleted in some or all lymphocyte lineages were important in identifying the lineage of the pregnancy-associated lymphocytes and providing insights to their functions. Mice lacking lymphocytes are immune deficient and must be housed under strict microbiological barriers to maintain their health. If this is done, most immune-deficient strains breed well and are excellent models for reproductive studies. Establishing and quantifying consistent phenotypes in the implantation sites of specific immune deficient mice provides powerful animal research tools in which reconstitution of implantation site structure can be assessed. These reconstitution approaches have many aspects that could be exploited in investigations of environmental toxicants. Four modeling approaches are briefly reviewed.

Model 1: Mice Genetically Deficient in Lymphocyte Lineages Have Quantifiable Implantation Site Defects. Mice deficient in NK cells were first reported in 1994–1995 (Wang et al., 1994; Di Santo et al., 1995). Our histological studies of implantation sites from NK cell-deficient strains, modified in different genes, showed that no uNK cells were present and that the architecture of implantation sites was disturbed. In particular, the decidual spiral arteries did not undergo the physiological changes of pregnancy and the decidua was hypocellular. To quantify these anomalies, vesse-to-lumen ratios and cell nuclei per square millimeter of decidua basalis are measured. Pregnancies proceed to term without evidence of dystocia. Litter sizes and weaning rates are normal (Greenwood et al., 2000).

To confirm that the absence of uNK cells was the key to the phenotype, reconstitution of the mice was undertaken, using marrow grafts from SCID (severe combined immuneodeficient) mice, a strain that produces no lymphocytes other than NK cells. When the grafted mice were mated, their implantation sites had high numbers of uNK cells and the spiral arteries and decidua matched those in normal pregnant mice (Guimond et al., 1998). This suggested that uNK cells produce molecules regulating gene expression in endothelium and smooth muscle cells of the decidual spiral arteries and in decidua. Interferon gamma (IFN-γ) is a key product of NK cells. Engraftment of alymphoid mice ($RAG^{o/o}$/$\gamma c^{o/o}$) with marrow from mice deleted for IFN-γ production reconstituted uNK

cell numbers but failed to correct the vascular and decidual anomalies (Ashkar et al., 2000). In contrast, treatment of pregnant alymphoid mice with IFN-γ gave complete normalization of the vessels and decidua in the absence of any uNK cells. This indicates a central importance for IFN-γ, a regulator of expression of hundreds of genes, in implantation site health. It also identifies a potential target molecule for monitoring of environmental contaminant effects on the uterine immune system.

Model 2: Monitoring Sites of Cell Precursors and Their Mobilization to the Pregnant Uterus *in Vivo*. Availability of animals having no ability to generate NK cells permits study of the origin of uNK cells by transplantation of tissues from mice with normal lymphocyte precursors. Uterine segment transplantation was used to show that the precursors do not reside in the uterus, while lymphoid tissue transplants to mated $RAG^{o/o}/\gamma c^{o/o}$ showed that all lymphoid tissue had at least some minor precursor cell content. Spleen cells, but only those harvested from donors who were in the early (gestation day [gd] 3 or 5) stages of pregnancy, were the richest source of uNK cell precursors (Croy et al., 2001). This has provided a model in which molecules responsible for homing patterns of cells can be assessed. Spleen cells are collected from gd 3 pregnant donors, genetically deleted for the genes of interest, and transplanted into mated alymphoid mice. Histological specimens from implantation sites are evaluated for numbers of uNK cells, their stages of maturity (granularity and cell diameter), and their distribution relative to the decidual spiral arteries in comparison to controls. Evaluation of environmental agents for interference with cell homing patterns to the uterus could be evaluated using this assay.

Model 3: Evaluation of Uterine Stromal Cell-Mediated Effects. A cDNA microarray analysis to identify IFN-γ-regulated genes in decidua basalis from normal mice at gd 6 and 10 (the genes potentially regulated by uNK cells) revealed large gains in expression of two members of the α^2-macroglobulin (α^2M) gene family. Products of this molecular family are found in high concentrations in blood plasma where they serve as protease inhibitors and cytokine transporters. Implantation sites from mice simultaneously lacking the two most highly expressed known members of the family, MAM and MUG-1 (Umans et al., 1999) were studied and found to have a phenotype related to that in NK cell-deficient mice. In $MAM^{o/o}/MUG\text{-}1^{o/o}$ mice, the spiral arteries dilated but were thick walled due to a cuff of trophoblast-like cells while decidua was hypocellular. In preliminary studies, infusions of human α^2M into NK cell-deficient mice promoted spiral artery dilation, but was less effective than infusions of IFN-γ (V:L ratios at gd 12 for PBS, αtwoM; and IFN-γ infusions were 2.8, 1.8, and 1.2, respectively; in normal mice, the V:L ratio is 1.2). A novel member of the αtwoM family, AM-X, was also identified and is being cloned. AM-X is expressed by stromal cells as they decidualize and by cells surrounding the spiral arteries. These studies highlight that uNK cells have regulatory actions on decidual cells that can be dissected and monitored.

It is also true that stromal cells act on uNK cells. Uterine stromal cells produce interleukin-15 (IL-15), the major survival signal for NK cells. Regulation of IL-15 in the uterus is unusual because it appears to be independent of the transcription factor IRF-1 used in lymphoid tissue (Croy et al., 2001). Mice, in which overexpression of genes such as IL-15 has been induced, are also valuable because overactivity of uNK cell behavior can be quantified.

Model 4: Use of Mouse Uterine and Lymphoid Tissues to Evaluate Human Cells. Tissue sections from mice can be used to assess functional interactions between endothelium and human lymphocytes *in vitro* under conditions that mimic shear flow within blood vessels (Frey et al., 1998). We found that pregnancy of the mouse donating the tissue enhanced this interaction. In peripheral lymphoid tissues, elevated adhesion was observed by gd 3, peaked at gd 6–8, and was sustained until birth. Postpartum adhesion levels were the same as in tissues from virgin mice. When adhesion of human blood lymphocytes, prelabeled with the NK cell marker CD56, was examined on uterine tissue sections from virgin mice, the $CD56^{bright}$ cell subset was enriched from 1.5 to 25 percent of the total cells. Further threefold enrichment occurred if the uterine sections came from pregnant mice. Antibodies to L-selectin or α4-integrin blocked adhesion. The dynamic changes in endothelium, demonstrated in these experiments, were induced equivalently in ovariectomized females treated by either estrogen or progesterone or both. The finding that steroid hormones change the functional properties of endothelium in a way that is recognized by lymphocytes has been consistent across 20 human lymphocyte donors using cell lines expressing single adhesion receptor molecules. This assay, in a modified form, could have applications in toxicology to monitor environmentally induced changes in endothelial cells within the uterus that would be recognized by human cells.

Acknowledgments. The studies reported include work done in collaborations with Dr. S.S. Evans, Roswell Park Cancer Institute, Buffalo, New York; Dr. F. van Leuven, K.U. Leuven, Leuven, Belgium; Dr. A.M. Hayes, University of Guelph, Guelph, Canada; and Dr. Michael Caligiuri, Ohio State University, Colombus, Ohio. The studies were conducted by research trainees in my laboratory and supported by grants from Natural Sciences and Engineering Research Council of Canada (NSERC), Ontario Ministry of Agriculture Food and Rural Affiars (OMAFRA), and Canadian Institutes of Health Research (CIHR).

REGULATION AND ASSESSMENT OF UTERINE CONTRACTILITY AND CERVICAL RIPENING DURING PREGNANCY

R.E. Garfield

The problems associated with labor during pregnancy are among the most important health issues facing health care providers of women. Understanding the regulation of the uterus and cervix in pregnancy and developing methods to

control their function are essential to solving problems relating to labor. We have evaluated uterine contractility and cervical ripening regulatory mechanisms during pregnancy in animal models and in humans under a variety of conditions. These studies suggest that environmental toxicants could alter uterine and/or cervical function and thereby accelerate or prolong the course of pregnancy. Our studies, in accordance with many others, suggest that both the uterus and the cervix pass through irreversible changes in preparation for normal-term labor as well as during preterm labor. The preparatory step in the uterus occurs just prior to labor and consists of an upregulation of systems important for augmented myometrial contractility, including increased cell-to-cell coupling, changes in ion channels, receptors for stimulants, and a downregulation of inhibitory systems such as nitric oxide. In the cervix and also in fetal membranes, the preparatory step during term delivery is temporally different from that in the uterus, indicating separate control systems. In the cervix the preparation step includes a slow progressive softening during the last half of pregnancy due to a decrease in collagen concentration prior to dilation, which occurs immediately before or accompanies labor.

Unfortunately, at the moment, we have only crude, inaccurate, and subjective methods to clinically measure the functional changes in the uterus and cervix that occur in preparation for labor. Without better diagnostic tools we may never advance our understanding of the uterus or cervix and may never find better treatments to modify their activity. In the past several years, we have developed noninvasive methods to quantitatively evaluate the uterus and cervix based, respectively, on recording of uterine electrical signals from the abdominal surface (uterine electromyography [EMG]) and measurement of light-induced cervical collagen fluorescence (LIF) with an optical device (collascope). The EMG method uses electrodes placed on the abdominal surface and systems for digitizing, recording, and analyzing the electrical bursts of the underlying uterine EMG activity which produce the contractile events. The system and methods are similar to recording cardiac electrical activity with electrocardiography. The collascope is a specially constructed device that illuminates a small area of the ectocervix and excites the collagen in the tissue to fluoresce. The fluorescent signal is analyzed by the device and is proportional to the amount of collagen in the cervix. Using an EMG labor monitor and collascope, we have examined more than 400 pregnant patients in the clinic and hundreds of animals in the laboratory. Both methods are rapid and allow accurate assessment of uterine contractility and cervical ripening. Our studies indicate that uterine and cervical functions can be monitored successfully during pregnancy using these approaches and that these techniques might be used in a variety of conditions associated with labor to better define management. The potential benefits of the proposed instrumentation and methods include quantitative procedures to predict the onset and progress of the preparatory steps leading to normal or preterm labor, reduction in the rate of preterm delivery, improvement in maternal and perinatal out-

come, monitoring treatments, decreasing cesarean section rate, and improved research methods to better understand uterine and cervical function.

FUNCTION OF STEROIDS IN PARTURITION AND PRETERM LABOR

William Gibb

Increased uterine contractility at term and preterm labor results from activation and then stimulation of the myometrium. Activation can be provoked by mechanical stretch of the uterus and by an endocrine pathway resulting from increased activity of the fetal hypothalamic–pituitary–adrenal (HPA) axis. Stimulation of the myometrium can result from a number of factors one of which is likely prostaglandins (PGs). The focus here is on the role of steroids in parturition, with particular emphasis on the regulation of prostaglandin production. In experimental animals, there is a decrease in the concentration of progesterone in the maternal plasma prior to the onset of labor, but in the human such a drop in progesterone concentrations does not occur. However, at the cellular level there are a number of similarities in the action of steroids in animal and human parturition in regulating PG formation and metabolism. In parturition the type 2 isomer of prostaglandin H synthase (PGHS-2) is particularly important and its expression has been found to increase in term and preterm labor. In fetal sheep, increased cortisol regulates PGHS-2 expression in the placenta in an estrogen-independent manner, resulting in increased levels of PGE_2 in the fetal circulation. Later increases in maternal uterine expression of PGHS-2 require estrogen and lead to increased concentrations of $PGF_{2\alpha}$ in the maternal circulation. In women, the fetal membranes surrounding the amniotic cavity are thought be an important source of prostaglandins involved in parturition. Formation of prostaglandins in these tissues is regulated by paracrine and autocrine mechanisms. In women, cortisol can contribute to increased PG production in fetal tissues through upregulation of PGHS-2 and downregulation of 15-hydroxyprostaglandin-D dehydrogenase (PGDH), the principal enzyme involved in the metabolism of prostaglandins. The effect of cortisol on chorion expression of PGDH reverses a tonic stimulatory effect of progesterone and likely occurs through the glucocorticoid receptor. By competing with progesterone inhibition, cortisol also increases expression of placental corticotropin-releasing hormone (CRH), which can in turn increase prostaglandin production. The $_{11}\beta$hydroxysteroid dehydrogenase, an enzyme which interconverts the active glucocorticoid, cortisol, and the inactive glucocorticoid, cortisone, appears to be important in regulating the effect of cortisol on prostaglandin metabolism in the placenta and chorion. In turn, this enzyme is upregulated in the fetal membranes by prostaglandins, forming a feed-forward (positive feedback) loop leading to increased prostaglandin production. Other agents such as pro-inflammatory cytokines similarly upregu-

late PGHS-2 and decrease expression of PGDH, indicating the presence of several mechanisms by which labor at term or preterm may be initiated.

CLINICAL AND PUBLIC HEALTH INTERVENTIONS—WHY NOTHING HAS WORKED

Robert L. Goldenberg

Conceptually, the origin of preterm birth can be divided into deliveries initiated by the clinician for the benefit of either the infant or the mother (indicated preterm birth) or those that follow either spontaneous preterm labor or spontaneous rupture of the membranes. The latter two categories taken together are often called spontaneous preterm birth. Approximately 20 percent of preterm births are indicated, approximately 30 percent follow spontaneous rupture of the membranes, and approximately 50 percent follow spontaneous preterm labor. The ultimate method of delivery, whether vaginal or by cesarean section, is not part of this definition.

Indicated preterm births usually occur because the mother is severely ill with a life-threatening condition, usually preeclampsia, or the fetus shows signs of deterioration and risk of fetal death, often in conjunction with maternal preeclampsia or fetal growth restriction. Attempts to reduce the prevalence of the conditions leading to indicated preterm birth, especially preeclampsia and fetal growth restriction, have generally failed. Examples of failures to reduce the severity or prevalence of these conditions include nutritional interventions, maternal bed rest, low-dose aspirin, and calcium supplementation. Because the prevalences of preeclampsia and fetal growth restriction appear to have remained unchanged, while there is increasing evidence that early delivery for fetal distress associated with these conditions leads to a reduction in stillbirths, the percentage of infants born following an indicated preterm birth seems to be increasing. It is emphasized that since the ultimate goal of obstetric care is to increase the number of living infants born without handicap, not to reduce preterm birth, this increase in preterm birth may, in fact, be beneficial. In any case, there is nothing on the horizon to suggest that a reduction in the prematurity associated with these conditions will occur in the near future.

Spontaneous preterm births are often divided into those that occur early and those that occur later. Early preterm births, those at less than 28 or 30 weeks' gestation, generally occur in association with an intrauterine infection or placental hemorrhage. In a study at our institution, 83 percent of the spontaneous preterm births weighing <1,000 grams were associated with bacteria in the fetal membranes prior to membrane rupture. Therefore, a chronic intrauterine infection with relatively low-virulence organisms such as ureaplasma, mycoplasma, and bacteroides is associated with and probably causal for most early preterm births. Later preterm births, especially those that occur at 35 and 36 weeks'

gestation, are, for the most part, not associated with infection, placental hemorrhage, or a specific etiologic factor. Instead, these preterm births appear to occur through the normal mechanisms responsible for term labor, which, however, occur earlier than usual. Women having a preterm birth at these gestational ages often have an increased number of risk factors, such as maternal thinness, smoking, and various psychosocial characteristics, but often have no specific precipitating cause for the spontaneous labor or rupture of membranes.

Interventions to reduce spontaneous preterm birth are categorized as targeted or general. General interventions might include providing prenatal care to a population that previously had none, improving the general quality of prenatal care, providing across-the-board nutritional supplementation, or providing some sort of social support or home visiting to a population of pregnant women. These types of interventions, likely because they are so nonspecific, have rarely been shown to be beneficial in reducing preterm birth.

Nutritional interventions deserve particular attention. Although preterm birth is clearly associated with maternal thinness, in developed countries neither nutritional counseling nor caloric, vitamin, or mineral supplementation has had much, if any, impact on the preterm birth rate. High protein nutritional supplementation has been associated with increased risk of preterm birth. These findings likely do not apply in developing countries where randomized trials of both vitamin or mineral and caloric supplementation have been associated with improved outcomes.

In recent years, the general trend for attacking preterm birth has been to identify specific risk factors and define an intervention to either eliminate or treat the risk factor in an attempt to reduce the associated preterm birth. Risk screening encompasses various demographic and medical history questionnaires such as that developed by Creasy et al. in the early 1980s, home uterine activity monitoring to determine an increase in uterine contractions, cervical ultrasound to demonstrate shortening of the cervical length, fetal fibronectin screening looking for a fetal protein in the vagina (where it should not normally occur), and a wide variety of other types of attempts to define populations at increased risk. Cervical or vaginal fetal fibronectin is probably the most potent risk factor, followed by a short cervical length. To date, however, despite a number of attempts, no intervention targeted to these risk factors has consistently been shown to reduce preterm birth.

As stated earlier, intrauterine infection is associated with a very large proportion of the earliest preterm births. Often, this intrauterine infection is linked to the presence of bacterial vaginosis, which, in nearly 20 studies, is associated with an approximately twofold increased risk of a preterm birth. Various attempts using antibiotics to treat either the bacterial vaginosis (BV) or the intrauterine infection thought to be associated with bacterial vaginosis have produced mixed results. In developed countries, if antibiotic treatment of BV actually works to prevent prematurity, it appears to work only in those women who have

had a prior preterm birth. Results from developing countries, such as in the Rakai region of Uganda, suggest that mass treatment with antibiotics of a population of pregnant women may reduce the preterm birth rate.

The use of antibiotics in women in early preterm labor in an attempt to reduce preterm delivery has more often than not been unsuccessful. There are, however, two randomized studies, both using metronidazole and ampicillin, that suggest benefit. Nevertheless, it appears that in women in preterm labor, the use of antibiotics alone is not likely to have a major impact on the early preterm birth rate. If infection exists and antibiotics do not seem to cure the infection and prevent the preterm birth, the question is, of course, Why not? Many potential explanations can be given, but it may be that once the intrauterine infection is established, antibiotics may actually increase the inflammatory response and hasten the premature delivery rather than delay it. An alternate explanation may be that the intrauterine bacteria are in an anatomic space, or in an environment such as a biofilm, that protects them from antibiotic treatment. There are many examples of a "structural" protection of bacteria from antibiotics in other parts of the body.

In summary, it is clear that at the present time, the interventions that have been applied generally to populations and to specific targeted high-risk populations have not achieved any real reduction in the preterm birth rate. The likely explanation is that the events leading to preterm birth are far more complicated than many of us realize and that, in general, we know far too little about the sequence of events leading to spontaneous preterm birth. Only when we better understand the biology of both early and late spontaneous preterm birth will we be likely to develop effective interventions.

ADVERSE CHILD OUTCOMES ASSOCIATED WITH PRETERM BIRTH

Maureen Hack

Technologic and therapeutic advances in neonatal intensive care have led to the improved survival of preterm infants. Whereas prior to the 1990s very few infants survived with birth weights less than 2 pounds, the vast majority of such infants now survive. The improved survival has however been associated with an increase in neonatal complications including brain injury and chronic lung disease, which are most prevalent among the smallest and least mature infants.

Neonatal complications of prematurity, including respiratory distress syndrome, chronic lung disease, brain hemorrhage, infections, and poor growth, result in health and developmental problems during childhood. These include an increase in respiratory infections and asthma, poor growth attainment, and neurological and developmental handicaps. The rates of cerebral palsy, poor vision, deafness, and mental retardation are higher among preterm than term-born chil-

dren. These impairments increase with decreasing gestational age and birth weight. Predictors of poor neurodevelopmental outcomes include the neonatal complications of prematurity as well as socioenvironmental risk factors. The same socioenvironmental factors that predispose to preterm birth usually continue after birth and may have deleterious effects on childhood health and development. The major neurodevelopmental impairments such as cerebral palsy and mental retardation can be diagnosed in early childhood. However, more subtle problems in behavior and functioning may present later at school age even among children who have no overt neurodevelopmental sequelae. These school age problems include poor visual motor and gross motor functioning, poor math abilities, behavioral problems mainly related to poor attention and hyperactivity, and social–emotional immaturity. Although respiratory and other health problems tend to diminish during childhood and catch-up growth occurs among many of the children, the school functioning problems do not resolve and persist into adolescence. More preterm than term-born children fail grades and fewer complete a high school education. Children born preterm have increased special health care needs compared to term-born children. During infancy and early childhood these needs pertain to both medical and educational services. However, during the school age and adolescent years they pertain mostly to special education, physical and occupational therapy, and counseling. Differences in intelligence between term and preterm children are evident even among preterm children who weigh between 3 and 5 pounds at birth and who do not require neonatal intensive care.

A FRAMEWORK FOR SOCIAL AND CULTURAL DETERMINANTS OF PREMATURITY

Carol J. Rowland Hogue

Although stressful events and lack of social support during pregnancy have been associated in some studies with increased risk of preterm delivery, interventions to increase social support have, in general, not lengthened gestation. Lack of effect may suggest that the causal hypothesis is faulty. Alternatively, the trials may have been diluted by inclusion of women in the intervention group who did not need social support. Some have argued that currently available interventions need to be offered only to women who both lack intimate support and are at high risk of preterm delivery. Other reasons for inconsistent results in observational studies and clinical trials may be incomplete stress exposure assessment or inaccurate delineation of the stress–health causal model, leading to measurement error and failure to account adequately for confounding and effect modification.

The classic "host, environment, agent" triangle of epidemiologic causality can illustrate what might be included in a more comprehensive test of the stress–

preterm delivery hypothesis. The host is the individual woman, the environment is her social and cultural context, and the agent is the immediate stressful event(s) requiring her response. This framework provides a context to determine the extent to which a given theory may include important and potentially interrelated factors.

Host Susceptibility. Individuals may differ in psychological and physiological responses to the same stressor, reflecting differences in the context within which the stressor occurs and differences in individual likelihood to respond to that type of stressor. Differential host susceptibility may be specific to the type of stressor. Potential risk factors for host susceptibility may include factors present at birth or related to personality, early life experiences, coping strategies, and circumstances of the pregnancy. In this framework, external resources available for coping with stress, such as levels of social support, are part of the environmental context.

Environments or Contexts. The social and cultural context of a pregnant woman may add to or ameliorate her level of distress. Ameliorating environmental factors include intimate social support, which has been associated with improved pregnancy outcome in a number of studies, although attempts to replicate social support in clinical trials to prevent poor pregnancy outcomes have not been effective. The chronic stress associated with environmental or social stressors may be measured at the individual level, for example, through inquiry into daily hassles as well as into perceptions of environmental issues. However, the negative health impact of environmental stressors is dependent not only on individual perception, but also on the actual environmental risks. For this reason, measurement of environmental risks must include contextual variables. Stressful enviromental factors include those associated with gender, with socioeconomic status, and with race or ethnicity.

Agents. Agents of stress are those events that provoke immediate individual response to a physical or emotional challenge. Stress is necessary for growth, development, and maintenance of brain and muscle activity. However, prolonged stress or an acute stressor that overwhelms the individual's ability to return to homeostasis can result in ill health. Stress results from host and environmental risks as well as acute stressful events. Whether the individual is overwhelmed depends not only on the strength of the agent but also on host susceptibility to stress, as well as the background level of environmental and contextual stress, as mediated by the background level of host, environmental, and contextual resources for handling stress.

Two kinds of stress agents have been studied extensively with respect to their impact on pregnancy outcomes. These are acute, stressful life events and hard physical labor—a type of individual, chronic exposure. A third kind of agent, individual experiences of racism, holds potential for explaining at least part of the excess preterm delivery rate among African Americans and the in-

creased preterm delivery rate among Mexican Americans who have lived in the United States for most or all of their lives.

Connections Among Host, Environments, and Agents. Host factors, environmental stressors, and acute stress triggers that have been hypothesized to increase women's risk of delivering prematurely do not operate in isolation, but are often interconnected. For example, the environmental risks of poverty and racism may operate through increasing host susceptibility by lowering self-esteem, lessening a sense of personal control, and increasing fatalistic views. To put together the many ways in which stressors and stress mediators may interact will require a unified theory of stress that includes not only various types of stress contagion but also the causal pathways for contextual stressors and other stressors. There is great need for better theoretical work in this area, coupled with a need to link epidemiologic theory development with biophysical markers of stress in studies of health outcomes.

CURRENT APPROACHES TO REPRODUCTIVE AND DEVELOPMENTAL TOXICITY TESTING AND RISK ASSESSMENT

Carole A. Kimmel

Approaches for reproductive and developmental toxicity testing of environmental chemicals as well as pharmaceutical agents include evaluations of fertility, pregnancy maintenance, parturition, lactation and survival, growth, and development of offspring. Rats and rabbits are the most frequently used animal models. Gestation length can easily be determined because the time of mating and the day of parturition are specifically recorded.

Testing protocols for reproductive and developmental toxicity evaluation of pesticides and industrial chemicals include evaluations of gestation length and of survival, growth, and development of offspring. Recent changes in the prenatal developmental toxicity testing protocol further increase the possibility of detecting alterations in gestation length. Treatment of pregnant animals was extended from the end of organogenesis to the day before termination in both rats (from gd 6–15 to gd 6–20) and rabbits (gd 6–19 to gd 6–28 or 29). Thus, effects of chemicals in late gestation on litter size, survival, growth, and development of the fetus, as well as on the occurrence of resorptions, abortions, or premature delivery in the females, would more likely be detected. When a developmental neurotoxicity study is conducted, typically in rats, the testing protocol includes exposure from early pregnancy throughout gestation and for part or all of the lactation period. Thus, animals are exposed until the end of gestation and gestation length is recorded, along with litter size, survival, and growth of the offspring. For the reproduction and fertility effects testing protocol, rats are typically used and are exposed from before mating and throughout gestation and

lactation. F_1 pups are continued on the same exposure level into adulthood, and then are mated, with the same level of exposure continuing throughout their pregnancy and lactation periods. Length of gestation is recorded for both parental and F_1 females, as are litter size, survival, growth, and development of the offspring.

Similar protocols are used by the Food and Drug Administration (FDA) for testing food additives and contaminants for reproductive and developmental toxicity. The standard testing protocols for pharmaceutical agents are designed to cover all stages of development, from the premating period through gestation and lactation, although long-term multigeneration studies are not typically conducted. Recently, pediatric evaluation of pharmaceuticals has gained a greater emphasis within the FDA. Testing guidelines have not been established as yet, but guidance on areas of concern is available, and includes the potential toxicity of drugs in premature infants.

The Environmental Protection Agency (EPA) risk assessment guidelines for developmental toxicity (U.S. EPA, 1991) and reproductive toxicity (U.S. EPA, 1996) discuss gestation length, premature delivery, and growth retardation of offspring and their consideration in hazard characterization as important endpoints of reproductive and developmental toxicity. Significant shortening of gestation can lead to adverse outcomes of pregnancy such as decreased birth weight and offspring survival. Several examples exist in the literature of agents that cause premature delivery in commonly used laboratory animal species, including mice, rats, and rabbits. Alterations in gestation length appear to be effects that occur at higher dose levels, with effects on offspring survival, birth weight, or other measures of growth and development as more sensitive indicators of reproductive and developmental toxicity.

ASSESSMENT AND RELEVANCE OF ENVIRONMENTAL CHEMICAL EFFECTS ON UTERINE MUSCLE

Rita Loch-Caruso

Although regulation of uterine contractility is fundamental for successful pregnancy, relatively little attention has been given to environmental chemical effects on the uterine muscle. The bulk of the uterine wall is comprised of the myometrial smooth muscle layer. During pregnancy, the myometrium increases substantially in size to accommodate the growing fetus and to prepare for the task of childbirth. With completion of the gestational term, the myometrium generates the forceful, oscillatory, repetitive, and coordinated contractions over a sustained duration that are necessary for successful parturition. Preterm development of such contractions by environmental chemicals could promote premature birth in the absence of successful intervention.

As in all muscle, sufficient elevations of myometrial intracellular calcium concentration initiate cell contraction. Gap junctions in the myometrium increase at parturition to form a communication network of intercellular channels that promote the coordination of contractions of individual myometrial cells. Although much diversity exists among species regarding the fetal and maternal signals that initiate parturition, mechanisms of parturition appear to converge at the level of the myometrium. Consequently, my laboratory has used *in vitro* experimental systems of myometrial cells and tissues of laboratory animal species to study direct toxicant actions in uterine muscle.

Uterine contractility responses are assessed by suspending in standard muscle baths uterine strips cut along the longitudinal axis of uteri from pregnant rats, and monitoring changes in the force and frequency of spontaneous oscillatory contractions in response to the test chemical. Using this approach, polychlorinated biphenyl (PCB) mixtures, lindane (γ-hexachlorocyclohexane), β-hexachlorocyclohexane, and various 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane DDT and PCB isomers were shown to exert rapid and direct actions on uterine contractions. By using biochemical, fluorometric, and pharmacological approaches in myometrial cell culture and tissue contractility experiments, elevation of intracellular calcium concentration via activation of voltage-operating calcium channels were linked to the stimulatory activities of Aroclor 1242. These results suggest that some environmental chemicals may directly stimulate uterine contraction by activating calcium-dependent mechanisms. In addition, prolonged exposure to the estrogenic PCB 4-hydroxy-2′,4′,6′-trichlorobiphenyl increased oxytocin-induced oscillatory uterine contraction frequency in an estrogen receptor-dependent manner, showing the potential for estrogenic environmental chemicals to stimulate uterine contractions via indirect mechanisms involving oxytocin.

Conversely, the ability of acute exposures of lindane and 4-hydroxy-2′,4′,6′-trichlorobiphenyl to abolish spontaneous oscillatory uterine contractions was associated with inhibition of myometrial gap junction-mediated intercellular communication. In the case of lindane, the inhibition of myometrial gap junction communication was via an oxidative stress-mediated mechanism. Whether activation of gap junction communication and alteration of cell redox are mechanisms of toxicant-induced stimulation remains to be investigated.

These laboratory studies provide insight into mechanisms by which environmental chemicals could stimulate preterm birth. In general, experimental toxicology best contributes to our understanding of risks to parturition from environmental chemicals when interpreted in conjunction with epidemiology findings, providing biological plausibility for epidemiological associations of exposures and outcomes as well as information on chemicals of concern for future epidemiology investigations.

EXPOSURES TO ENVIRONMENTAL AGENTS AND PRETERM DELIVERY

Matthew P. Longnecker

Data regarding occupational and environmental agents in relation to the risk of preterm delivery in humans were identified in the MEDLINE database using the PubMed search engine. The reference lists of articles identified were searched for additional reports. For several exposures the data suggested that environmental factors contribute to the risk of preterm delivery.

In three studies of air pollution effects, including one from the United States, particulate matter (either total suspended particles or particulate matter less than 10 microns in diameter) was associated with a modest increase in risk of preterm delivery; suggestive data also implicated sulfur dioxide. All three studies had ecologic–time series type designs, employed a limited number of air monitoring stations, and had little personal-level data.

In a recent report about a United States population first studied in the 1960s, maternal pregnancy serum level of the 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane DDT metabolite DDE was associated with increased risk of preterm birth. The association was also seen in some earlier, smaller studies, but the exposure levels were lower. Whether the association is causal has not been resolved, although if it were causal, the effects are probably evident at levels of exposure seen only in countries where DDT has been used recently.

Potential risk factors for preterm birth identified in more than one occupational study were some maternal occupation (such as metal, electrical, janitorial, food service, textiles); maternal occupational exposures (e.g., solvents); some paternal occupation (such as food service, textiles); and paternal occupational exposures (pesticides, e.g., atrazine in one study). As in other occupational studies of preterm birth, only very general information about exposure was available.

Data from a recent ecologic study suggest that high levels of arsenic in drinking water increase preterm birth. The association with arsenic was in a population with water arsenic levels that were about two times higher than those in high-exposed areas of the United States (e.g., selected counties in Utah).

Risk factors that have been studied and appear not to be associated with increased risk of preterm birth were chlorinated water disinfection by-products and use of video display terminals. Data regarding maternal lead exposure were inconsistent, though findings in the largest prospective studies that adjusted for multiple confounders do not support a relation.

While many of the potential factors studied so far merit further investigation, additional factors for which data were considered inadequate were cadmium, paternal occupational lead exposure, polychlorinated biphenyls, and various occupational chemical exposures among mothers and fathers.

None of the factors presented are established as related to risk of preterm

delivery. Strengths and weakness of the review strategy will be discussed as will priorities for future research directions.

FETAL SIZE AND PRETERM BIRTH

Stephen J. Lye

The onset of labor requires both the *activation* and the *stimulation* of the myometrium to generate the intense and coordinated contractions needed to bring about the delivery of the neonate. Activation involves a switch in the contractile state of the myometrium from a state of inactivity to one in which the muscle develops increased excitability, increased responsiveness to uterotonic agonists, and enhanced intercellular communication. Once *activation* is induced, *stimulation* is achieved through the increased production of agonists such as stimulatory prostaglandins and oxytocin. We have predicted and have gained evidence to suggest that the process of myometrial activation results from the synchronous increased expression of a cassette of genes encoding "contraction-associated proteins," or CAPs, including ion channels (which regulate the resting membrane potential of myocytes and hence their excitability), agonist receptors (to enhance myometrial responsiveness to stimulatory hormones), and gap junctions (to provide for enhanced cell–cell communication and therefore synchronization of uterine contractions). Clearly, knowledge of the mechanisms that control the process of myometrial activation is central to the development of strategies to prevent preterm labor.

We present evidence that myometrial activation and the onset of labor is ultimately controlled by the fetal genome through two pathways—one involving fetal endocrine signals and the other mediated by mechanical signals induced by stretch of the uterine wall by the growing fetus.

The contribution of fetal endocrine signals in the onset of labor was first demonstrated by Liggins some 30 years ago. This pathway involves activation of the fetal hypothalamic–pituitary–adrenal axis leading to increased synthesis and release of cortisol from the fetal adrenal gland. Cortisol in turn induces the expression of PGHS-2 in the fetal placental, which leads to the production of PGE_2. As PGE_2 levels rise there is an induction of P450C17 activity, which in most species ultimately leads to the metabolism of progesterone through to estrogen. We have shown that estrogen positively regulates the expression of CAP genes, while progesterone suppresses their expression. The mechanisms by which these steroids act remains to be determined although there is evidence to suggest that transcription factors of the AP-1 family contribute to the activation of CAP genes. In contrast to virtually all other species, progesterone levels do not fall at term in humans; nevertheless there is evidence to suggest that progesterone is required for the maintenance of pregnancy in women. It is likely that some mechanism exists in humans to block progesterone signaling at term and there-

fore induce a functional withdrawal of progesterone, permitting the activation of CAP genes, myometrial activation, and the onset of labor.

Although this endocrine pathway appears to be necessary, data we obtained several years ago suggested that this pathway is not, in itself, sufficient for the initiation of labor. In studies in unilaterally pregnant rats we found that even though the endocrine changes at term occurred normally, CAP gene expression increased only in the gravid horn and not the empty horn. However, if a plastic tube was placed in the lumen of the empty horn to induce stretch, the increase in CAP gene expression occurred to the same extent as in the gravid horn. These data suggested that both endocrine and mechanical signals (as a result of fetal growth) are required for the initiation of myometrial activation and the onset of labor. This led to the question of how pregnancy could be maintained to term if fetal growth led to increased CAP gene expression. As a result of a series of studies we now believe that during pregnancy, tension on the uterine wall leads to growth of the uterine wall, which in turn reduces the tension and thus prevents premature activation of CAP gene expression. This stretch-induced growth of the myometrium results from a hypertrophy of the myocytes and requires the presence of progesterone. The mechanisms by which myometrial cells sense that they are under tension and are able to transduce this into a signal that leads either to genes controlling myocyte hypertrophy or to myometrial activation remain to be determined, though our evidence suggests an involvement of the focal adhesion signaling through mitogen-activated protein kinsase (MAPK) and increased AP-1 protein expression.

Although there are many pathways by which preterm labor might be induced, increased myometrial contractility is likely common to all of them. We suggest that, at least for idiopathic preterm labor, premature activation of the endocrine and/or mechanical pathways contributes to preterm birth. It is well established that fetal hypoxia (such as might be induce by placental insufficiency) can lead to increased activity of the fetal HPA axis leading to increased cortisol synthesis. Initially negative feedback pathways would block further elevations in this steroid, although chronically elevated cortisol might negatively impact fetal growth. Continued hypoxic stimulation would eventually lead to cortisol induction of placental endocrine changes, myometrial activation, and the initiation of preterm labor with the birth of a baby that is small for its gestational age.

Multifetal pregnancies are known to be at increased risk of preterm birth. We believe that our data on stretch-induced increase in myometrial CAP gene expression offers a possible explanation for this observation. While the increased tension in the uterine wall would lead to increased myometrial growth there may be some limits to this capacity. At the very least, the stress placed on the mechanical pathway would likely make such pregnancies more susceptible to preterm birth from other causes. This model also suggests that large babies or pregnancies complicated by increased intrauterine volume (e.g., polyhydramnios)

might be at risk of preterm birth through premature activation of mechanical signals.

Recent clinical data from the group of Dr. Robert Gagnon (University of Western Ontario, Canada) supports a role for both the endocrine and the mechanical pathways in the onset of preterm birth. These data showed a twofold increase in the incidence of preterm birth in babies who were larger (>97 percentile) or smaller (<3rd percentile) than those who were appropriately grown.

SPECIES DIFFERENTIATION AND ANIMAL MODELS OF PARTURITION

Peter W. Nathanielsz

Here is a **working** definition of parturition: *Parturition is a multifactorial process that involves fetal, placental, and maternal mechanisms. Parturition involves the recruitment of interactive positive feed-forward loops and the removal of pregnancy maintenance mechanisms. Changes in these stimulatory and inhibitory mechanisms exhibit critical tissue-specific time relationships to each other.*

There are four central features in this definition.

Parturition

1. is a multifactorial process,
2. recruits interactive positive feedforward loops,
3. involves the removal of pregnancy maintenance mechanisms, and
4. mechanisms have critical time-dependent tissue-specific interrelationships.

The three indispensable processes involved in normal labor and delivery are

1. A switch in myometrial contractility pattern from contractures to contractions,
2. Rupture of the fetal membranes, and
3. Dilation of the cervix.

The Fetal Role in the Determination of the Duration of Pregnancy. In sheep, interruption of the fetal hypothalamic–pituitary–adrenal axis at any level will prolong pregnancy (Challis et al., 1994; Nathanielsz, 1996). Infusion of adrenocorticotropic hormone (ACTH) or cortisol to the ovine fetus will shorten the length of pregnancy. Taken together, these studies have been interpreted as indicating that the fetus determines the length of ovine pregnancy. However, while demonstrating that the fetus is involved in determining the duration of

pregnancy, each of these studies shows only that these fetal endocrine tissues are involved in the promotion of parturition.

Since parturition is a multifactorial system, it is difficult to determine precisely what is the **initiator** of the cascade that leads to delivery. In one very real sense, the signal for parturition is fertilization. Following fertilization, a sequence of genetically programmed events is set in train. This sequence unfolds in a manner and at a speed determined by both the genome and the environment, nature and nurture. As with most biological processes, though not all, the duration of pregnancy is *gene influenced, not gene determined.* In closely inbred groups of animals maintained under carefully regulated nutritional and environmental conditions, the duration of pregnancy is very homogeneous. This suggests that in such precisely defined environments, the fetal developmental steps that regulate the system are very precisely regulated by the genome. However, external stresses, nutritional challenges to the fetus, and other conditions can alter the duration of pregnancy by speeding up or interfering with one or more of the normal mechanisms.

A very pronounced example of this is the prolonged pregnancy induced when pregnant sheep ingest the corn lily on the fourtheenth day of pregnancy and expose the developing fetus to the alkaloid toxin 2-deoxyjervine, resulting in a cyclopian deformity and distorted brain development. Another good example is human anencephaly. There is a high incidence of polyhydramnios in anencephaly. When polyhydramnios occurs, the uterine muscle is stretched and premature labor is common. However, when anencephaly is not accompanied by polyhydramnios, human pregnancy is prolonged. These observations suggest some similarity between the neuroendocrine involvement in parturition in the sheep described below and the mechanisms that are critical to labor and delivery in human pregnancy (Nathanielsz, 1996).

Because of the inability to perform carefully controlled, invasive studies in human pregnancy, it is necessary to conduct studies in animal models, especially nonhuman primates. This presentation compares the evidence of fetal involvement in rhesus monkey pregnancy and sheep pregnancy.

Evidence for increased fetal adrenal function in late gestation. There is a remarkable similarity in the rise of fetal cortisol in the sheep and fetal androgen in the fetal monkey over the final 30 percent of gestation (Challis et al., 1994; Nathanielsz, 1996). This and other observations have led us to hypothesize that the similarity between nonhuman and sheep pregnancy lies in the rise in estrogen production prior to labor.

In sheep, fetal cortisol stimulates placental conversion of progesterone to estrogen (Anderson et al., 1975). In primates the placenta is an incomplete steroidogenic organ and estrogen synthesis by the placenta has an obligate need for androgen precursor (Novy et al., 1981).

Evidence for increased estrogen production in late gestation. Longitudi-

nal measurement of maternal estrogens immediately before spontaneous term labor shows an increase in all primate species studied in a detailed fashion.

Patterns of myometrial activity that occur throughout gestation. In all mammalian species studied to date, myometrial activity throughout pregnancy is of the contractures type, exemplified by long-lasting, low-frequency epochs of activity that have a very different temporal and amplitude pattern from contractions (Nathanielsz, 1996). At labor and delivery, contractures must switch to contractions to produce efficient delivery of the fetus. In the sheep this switch occurs once, generally at nighttime, and the ewe proceeds to delivery. In the monkey this switch occurs and augments for a few hours each night until delivery occurs after several switches (Nathanielsz, 1996).

Initiation of premature labor in rhesus monkeys by stimulation of estrogen production. *1. Estrogen as the central mediator of parturition mechanisms***:** In a series of studies in the pregnant rhesus monkey we observed that situations which resulted in elevated maternal estrogen concentrations in late pregnancy were generally accompanied by a switch in myometrial activity from the contractures to the contractions mode. These include following laparotomy and fetal catheterization surgery (Nathanielsz et al., 1984), during hypoglycemia induced by food withdrawal (Binienda et al., 1988), predelivery (Taylor et al., 1983), and following androstenedione administration to the pregnant monkey (Figueroa et al., 1989; Mecenas et al., 1996; Nathanielsz et al., 1998). Novy and colleagues were unable to induce premature delivery in the pregnant rhesus monkey by the administration of estrogen (Novy et al., 1983). We therefore hypothesized that under normal circumstances, estrogen exerts both paracrine effects at its site of generation from androgens and classical endocrine effects. Thus, we carried out androgen infusion to ensure that estrogen was produced at its normal locus of production.

2. *Infusion of androgen into the pregnant rhesus monkey to elevate estrogen biosynthesis***.** Continuous infusion of androstenedione into the pregnant rhesus monkey at 0.8 of gestation produces labor associated with the three indispensable processes described above: a switch of myometrial contractures to contractions, rupture of the fetal membranes, dilation of the cervix, and delivery of live young (Nathanielsz et al., 1998).

It is interesting to note that although the infusion of androgen was continuous and the maternal plasma estrogen concentration was elevated throughout the 24-hour day, the switch from contractures to contractions occurred only around the hours of darkness. This rhythm in the switch is likely to be driven by oxytocin since the switch can be abolished by oxytocin antagonists such as Atosiban.

3. *Inhibition of the effects of androstenedione by aromatase inhibitors***.** The proof that local estrogen production is key to labor and delivery is furnished by studies in which the promotion of labor by infusion of androstenedione is inhibited by aromatase inhibitors (Nathanielsz et al., 1998).

4. *Failure of estradiol infusions to produce premature delivery in the rhesus monkey:* Systemic infusion of estrogen does not precipitate labor and delivery in rhesus monkeys. This finding further supports a role for local paracrine actions of estrogen (Novy et al., 1983).

The Mother Determines the Precise Time That Labor Begins. We have demonstrated that the switch from contractures to contractions is regulated by maternal oxytocin acting on a myometrium that has been prepared by the rise in maternal estrogens described above. Firstly, the switch can be initiated prematurely by infusing androgen into the pregnant rhesus monkey to raise maternal estrogen. In this experimental paradigm, the switch is prevented if aromatase inhibitors are administered with the androgen to inhibit estrogen production (Nathanielsz et al., 1998).

Oxytocin antagonists will inhibit both the normal term switch from contractures to contractions and the switch that is produced prematurely by androgen infusion. Maternal plasma oxytocin concentrations in late gestation show a pronounced 24-hour rhythm with a peak in the early hours of the evening, coincident with the switch from contractures to contractions. A similar rhythm has been shown in pregnant women in late gestation. It is now clear that the failure to demonstrate a rise in oxytocin in late human pregnancy in previous studies can be attributed to a failure to obtain blood samples at the correct time of day.

The Role of Prostaglandin Synthesis, Degradation, and Receptor Activation. Prostaglandins have been shown by many investigators to be essential for the normal completion of parturition. Various animal models have been used to highlight the changing role of prostaglandins. Since these regulatory compounds work at the paracrine and autocrine level, it is essential to compare effects both in the whole animal and in vitro. Our recent studies have dealt with PG synthesis in the pregnant sheep and baboon and with changes in the various PG receptors in different intrauterine tissues throughout the last third of gestation and in labor in the sheep and baboon (Smith et al., 1998, 2001a, 2001b, 2001c).

TOXIC SOCIAL ENVIRONMENT: A FACTOR IN PRETERM BIRTH?

Janet Rich-Edwards

Two of the largest and most intractable risk factors for preterm birth are maternal social class and race or ethnicity. Attempts to "unpack" class and race into specific risk components have failed to explain more than a fraction of their predictive power. New approaches to the conceptualization and measurement of psychologic and social stressors should yield more insight into the elevated risk of preterm birth that accompanies poverty and minority status in the United States. Approaches to measuring maternal stressors and stress responses during

pregnancy are being refined. Previously neglected stressors, such as maternal experiences of violence and racism, are being addressed in new studies. The measurement of women's social position is being refined. New models of the interaction between psychosocial stress and physiologic pathways to prematurity are being tested, including potential associations of maternal stress with CRH levels and risk of infection. Finally, there is growing acknowledgment that chronic stress before pregnancy may be at least as important to pregnancy outcome as stressors occurring during pregnancy. As the science of measuring psychosocial predictors of health grows more sophisticated, we may find new explanations for the long-standing social and ethnic gaps in risk of preterm birth in the United States.

CAUSES AND MECHANISMS OF PREMATURE LABOR

James M. Roberts

This presentation reviews the currently popular theories of preterm labor to provide insight as to where toxicants could act to lead to preterm birth. In addition, it considers the fact that almost one-fifth of preterm births are iatrogenic, usually secondary to indicated delivery for preeclampsia, making preeclampsia another possible target for toxicants to increase preterm birth.

Currently, two theories hold most interest for the genesis of preterm birth. The inflammatory theory of preterm birth has received most attention. Another hypothesis that has received less attention is the relationship of preterm birth to abnormal implantation. One other hypothesis considered here, which had long guided thinking about preterm birth, is that preterm labor is merely term labor occurring early, essentially an abnormality of the biological clock.

The hypothesis that inflammation might contribute to preterm birth was originally stimulated by the concept that infection, more specifically subclinical infection, might lead to preterm birth. The association of bacterial colonization of fetal membranes with early spontaneous preterm birth, but not induced preterm birth, provided strong evidence for this hypothesis. The association of intraamniotic bacteria and inevitable delivery provided further support. The role of bacteria was biologically plausible since these organisms have the capacity to activate phospholipase and generate prostaglandins implicated in uterine contractility. It soon became evident that increased cytokines in amniotic fluid, even without microorganisms, were an excellent predictor of preterm birth. This raised the possibility that these cytokines, in addition to (or rather than) microorganisms, might be responsible for the activation of labor mechanisms. The concept that cytokines could be the important mediators raised the possibility that causes of inflammation, other than infection, could be involved in the genesis of preterm birth. The relationship of distal inflammatory stimuli such as periodontal infections was made plausible by this mechanism. In addition, the relationship of

preterm birth to maternal stress was suggested to be secondary to activation of the inflammatory response by CRH, for example. Cytokines could also be released by immune interactions between mother and infant. Furthermore, the recognition that even normal pregnancy is associated with a marked activation of the inflammatory response suggests that some women with preterm birth may simply be at the wrong end of the normal distribution of pregnancy-induced inflammatory responses. Environmental toxicants could also augment this inflammatory response.

Preterm birth is also associated with abnormal implantation. This is indicated by studies of placental bed biopsies, and placental pathology and by the increased frequency of growth restriction in infants with spontaneous preterm birth. This suggests similarities with other abnormal implantation disorders such as habitual abortion and preeclampsia. Although neither relationship has been extensively explored, we have found that as with preterm birth, early-onset preeclampsia (indicated delivery prior to 34 weeks) is associated with an increase of the acute-phase reactant feritin at 16 to 19 weeks' gestation. In addition, women who have had early-onset preeclampsia in their first pregnancy deliver in their subsequent "normal" pregnancy on average one week earlier than women with a normal first pregnancy. Information on control of human implantation is increasing rapidly and suggests targets for toxicants that lead to preterm birth.

Preterm birth is recurrent, and increased risk is inherited and varies with race. Although part of this increased risk can be environmental it is also likely that this is genetically influenced. In years past the impact of genetics was used to support the possibility that in some individuals the biological clock determining the onset of labor was abnormal. Much effort was directed at understanding term birth with the hope that this information would extrapolate to preterm birth. Distal mechanisms are undoubtedly similar and the understanding of term labor is pertinent to these. However, current thinking has largely abandoned the concept of an abnormal biological clock mechanism in favor of distinct factors stimulating term and preterm birth. Our level of understanding the mechanisms of preterm labor, however, does not justify abandoning this (or any) concept. The genetic contribution to preterm birth could also be contributions to abnormal implantation or inflammation. Especially pertinent to this workshop, interaction of environmental toxicants and genetically altered metabolism could contribute to preterm birth through any of the suggested mechanisms.

Preeclampsia accounts for 15 percent of preterm births. As a pregnancy-specific disorder that puts maternal and infant well-being in jeopardy, delivery is the only known effective treatment. In about 10 precent of cases this will necessitate preterm delivery. Preventing preeclampsia would prevent these preterm births. Mechanistic studies of preeclampsia have made considerable progress in the last 10 years. Large clinical trials testing one of the postulated pathogenic mechanisms, oxidative stress, are about to begin. Preeclampsia is also associated with exposure to environmental toxins. Women working in occupations with

increased exposure to organic solvents have a fourfold increased risk of the disorder. Anecdotally, in the portion of Mexico City with the most severe air pollution, preeclampsia–eclampsia accounts for 80 percent of maternal deaths.

Preterm birth is the major pregnancy problem in developed countries. There has been little progress in reducing the frequency of the disorder. It is quite probable that all preterm labor is not from a single cause and that all of the currently suggested precursors of preterm birth (and likely others) are important in different women and may be interactive. Understanding how environmental exposures may act upon these mechanisms could provide useful insights for prevention.

MOLECULAR MECHANISMS AND CELLULAR SIGNALING PATHWAYS ASSOCIATED WITH PARTURITION

Barbara M. Sanborn

Prior to parturition, there is a transition period during which a series of coordinated events prepare the uterine smooth muscle (myometrium) to respond to contractant signals and to be less responsive to relaxant signals. These events include alterations in the concentration of the many proteins that constitute components of the signaling pathways involved. During parturition, maternal and fetal compartments express the signaling pathways and produce the molecules that stimulate the myometrium to contract. The regulation of myometrial contraction is of paramount importance for the maintenance of pregnancy and for parturition. Understanding this regulation involves delineating the pathways that control contraction and relaxation and defining their interaction.

In myometrium, an increase in intracellular calcium (Ca^{2+}_i) favors contraction. The major contractant pathways, including those stimulated by oxytocin, target activation of phospholipase C (PLC), resulting in release of intracellular calcium from intracellular stores by inositol 1,4,5-triphosphate(IP_3) (Sanborn, 2001; Sanborn et al., 1998). Signaling pathways that activate PLC can also stimulate calcium entry through calcium release-activated channels, either directly or indirectly. The introductory calcium level (Monga et al., 1999) Ca^{2+}_i is lowered by the actions of plasma membrane and sarcoplasmic reticulum calcium pumps.

Agents that stimulate cyclic adenosine 5-monophosphate (cAMP) inhibit myometrial contractile activity, contractant-stimulated phosphatidylinositide turnover, and increases in Ca^{2+}_i (Sanborn, 2001; Sanborn et al., 1998). A major point of cAMP inhibition is at the level of PLC. Although $PLC\beta_1$ is not a substrate for cAMP dependent protein kinase (PKA), $PLC\beta_3$ is phosphorylated by this enzyme, as well as by cGMP-dependent protein kinase and protein kinase C (Yue et al., 1998, 2000; Xia et al., 2001). Myometrial plasma membranes possess A-kinase-associated-proteins (AKAPs), that serve to localize PKA there (Dodge et al., 1999a). The PKA that inhibits PLC in myometrial plasma mem-

brane appears to be associated with AKAPs, both in human cells and in pregnant rat tissue. The ability of activated PKA to inhibit the phosphatidylinositide turnover pathway is markedly diminished at term in the rat. This change is accompanied by a loss in PKA both from the plasma membrane and associated with AKAP150 (Dodge et al., 1999b). These changes are not accompanied by a change in AKAP expression in the membrane. Rather, there is a change in the ratio of PKA and protein phosphatase 2B (PP2B) associated with the plasma membrane and with AKAP150**.**

These data point to an important new mechanism, namely a change in the localization of AKAP-associated PKA in plasma membrane that may serve as the final regulatory checkpoint on the inhibitory action of cAMP–PKA on contractant-stimulated PLC activity. Recent evidence suggests that this change is correlated with the timing of parturition in the rat and that hormones play a role. Understanding the control of the scaffolding mechanism and its relevance in primates will be critical to understanding the actions of the agents utilizing this pathway to promote uterine relaxation. Such mechanisms may play a critical role in controlling the transition from myometrial relaxation to contraction.

Note this work was supported by HD09618 and HD38970.

BEHAVIOR, NUTRITION, INFECTION, AND STRESS: EPIDEMIOLOGIC CLUES TO THE STUDY OF THE ENVIRONMENT AND PRETERM BIRTH

David A. Savitz

To help focus the study of environmental agents that might influence the risk of preterm birth, the extensive literature on nonenvironmental factors warrants scrutiny. Epidemiologic research on a range of behaviors (tobacco use, cocaine use, physical activity), nutrition (iron, folate), infection (bacterial vaginosis), and psychological stress has been conducted for some time and offers insights into methodologic challenges, suggests possible etiologic pathways, and identifies potential confounding factors that must be considered. At present, strong predictors of preterm birth are limited to multiple gestation, prior preterm birth, and African-American ethnicity; weaker but modifiable influences include infection, tobacco use, low prepregnancy weight, lower socioeconomic status, and other prior adverse pregnancy outcomes. Despite extensive research, the roles of nutrition, cocaine use, physical exertion, and psychological stress remain unresolved. Nevertheless, these efforts raise questions and suggest approaches relevant to the study of environmental contributors.

1. The diversity of identified predictors of preterm birth—namely, multiple gestations, prior preterm birth, tobacco use, bacterial vaginosis, low prepregnancy weight, African-American ethnicity, and economic deprivation—suggests a

multiplicity of pathways, each making small, probabilistic contributions to the same endpoint. The relevant model seems to be one of multiple contributing factors operating through diverse mechanisms rather than a single cause or even a single pathway. The exploration of environmental contributors may expand the array of contributing factors, but there is no expectation that "the cause" of preterm birth will be found or isolated in the environment.

2. Influences on preterm birth have been more difficult to identify than causes of reduced birth weight. The predictors of reduced birth weight are often shared with predictors of preterm birth, but often have weaker associations. Such predictors of preterm birth as socioeconomic status and tobacco use seem to have strong influences on birth weight but only modest influences on preterm birth, indicating that the pathways probably overlap to some extent but are not identical. Direct examination of preterm birth, low birth weight, and small-for-gestational-age deliveries suggests modest overlap, such that synthesizing the literature requires considering research subsets defined by the pregnancy outcome under examination. One reason for the more limited success in the study of preterm birth may be the markedly greater uncertainty in measurement of duration of gestation as compared to birth weight, reflected in greater magnitude of error in vital records, lower quality of maternal reports of gestational age as compared to birth weight, and the more limited availability of gestational age from less developed countries. Duration of gestation is usually based on the last menstrual period, a fallible marker of the time of ovulation, although widespread use of ultrasound for dating has been helpful in increasing accuracy in many settings, a benefit to those studies that can incorporate this information over those based solely on last menstrual period.

3. Isolating specific causal agents from nonspecific influences of a healthful life-style and favorable socioeconomic conditions has been very difficult. As for many health endpoints, those who have a range of favorable attributes have more favorable outcomes. However, the lack of specificity in what aspects of "favorable circumstances" are responsible has been very challenging to researchers addressing preterm birth. For example, there are consistent findings of an association between cocaine use and preterm birth, reduced risk associated with leisure time physical activity during pregnancy, reduced risk with favorable nutritional status and use of prenatal vitamins, and increased risk associated with physically demanding occupations, yet none are necessarily causal, and isolating a true etiologic effect from a spurious association due to other unmeasured or unknown factors has been unsuccessful thus far. Isolating environmental agents from the circumstances that give rise to exposure will pose a serious challenge for the many environmental agents associated with socioeconomic deprivation and less favorable life-styles.

4. Preterm birth is clearly a heterogeneous entity, with distinctive contributing pathways, yet the most useful approach to subdividing the outcome for identification of etiologic factors is unclear. Many studies separate spontaneous from

medically indicated preterm delivery, a reasonable strategy, yet the common indications for medical intervention and early delivery (fetal growth restriction, hypertension) are also independent risk factors for spontaneous preterm birth. Some risk factors may be shared across spontaneous and indicated preterm births, whereas others are likely to differ. Other divisions of preterm birth are based on clinical presentation (idiopathic preterm labor, preterm premature rupture of membranes) or on severity of prematurity defined by duration of gestation. More novel approaches to categorization consider the underlying etiologic process (e.g., infection or inflammation, vascular compromise). There are considerable logistical and conceptual challenges to refining the endpoint for epidemiologic study.

5. Biological markers of exposure have much to offer since true prospective studies beginning early in pregnancy allow for assessment in the etiologically relevant time interval. Biological markers of nutritional status (serum folate, ferritin, transferrin receptor saturation), stress (corticotropin-releasing hormone, cortisol), tobacco use (urinary and serum cotinine), cocaine use (cocaine and benzoylecognine in hair and urine), and infection or inflammation (fetal fibronectin, cytokines) have all been incorporated into epidemiologic studies of preterm birth to great benefit. Participation in prenatal care offers unusually favorable opportunities to incorporate collection of biospecimens in large clinically based populations as opposed to community samples, with clear applicability to the study of environmental agents.

The study of environmental agents in relation to preterm birth is at a very early stage of development, with far less interest in the past than in potential environmental contributors to pregnancy loss or male infertility, for example. It is important as these research avenues move forward to ensure that sophisticated environmental approaches are combined with rigorous evaluation of the reproductive health endpoints and potential confounding factors, generally requiring a multidisciplinary research team.

GENE–ENVIRONMENT INTERACTIONS AND PRETERM DELIVERY

Xiaobin Wang

Although the causes of preterm delivery remain unclear, preterm delivery appears to be a highly complex entity determined by multiple environmental and genetic factors, as well as gene–environment interactions. Most previous studies have focused on socioenvironmental or clinical variables. The role of genetic susceptibility and gene–environment interactions in relation to preterm delivery is largely unexplored. This presentation provided a brief overview of gene–environment interaction. Then, it summarizes findings of gene–environment interac-

tions on preterm delivery from our ongoing molecular epidemiologic studies of preterm delivery in both Chinese and U.S. populations, specifically, interactions between maternal metabolic genes and benzene exposure and interactions between maternal metabolic genes and cigarette smoking. In addition, important methodological issues in this research field were discussed (see chapter 4 of this summary for more details). Our data have shown a consistent evidence of gene-environment interactions in diverse populations. However, more studies are needed and multidisciplinary collaborations are required in order to jointly and comprehensively assess the role of environmental factors, genetic factors, and gene–environment interactions in preterm delivery among populations with marked differences in social status and environmental exposures.

ROLE OF NITRIC OXIDE IN UTERINE ACTIVITY AND PREMATURE PARTURITION

Chandrasekhar Yallampalli

Preterm labor and delivery remain an important problem in obstetrics, with prematurity contributing to 8–10 percent of neonatal deaths and responsible for 60–70 percent of neonatal morbidity in the United States. There is no effective treatment for preterm labor. Most commonly used tocolytics, β_2-adrenergic agonists such as ritodrine and terbutaline, do not prolong pregnancy or improve neonatal outcome and have serious side effects. Nitric oxide has been studied in the last eight years as a potential tocolytic. This presentation examines the studies on (1) synthesis of NO and expression of NO synthase enzymes in the uterus of various species; (2) effects of NO on uterine relaxation; (3) regulation of NO synthesis and effects of NO on the uterine relaxation during pregnancy and labor; (4) effects of NO donors on preterm labor and possible prolongation of gestation; and (5) interaction of NO with other uterotonins in modulating uterine activity during pregnancy and preterm labor.

Nitric oxide is a simple, but highly reactive, endogenous chemical, hitherto known as an environmental toxicant. Since the first description of the therapeutic use of glyceryl trinitrate (GTN) appeared in 1879, organic nitrites have remained the cornerstone for the treatment of angina pectoris. GTN has been used in obstetrics as a uterine relaxant in cases of breech extraction. In 1993, we and others reported the presence of the NO system in the uterus of a variety of animals, suggesting that endogenous NO synthesized in the uterus could play a role in uterine quiescence during pregnancy. Morphological and biochemical studies of uterine samples demonstrated the presence of the NO system in the rat, rabbit, mouse, sheep, and human. Several studies demonstrated increases in NO synthesis in the uterus and in NO-induced uterine relaxation during pregnancy. Both NO synthesis and uterine sensitivity to NO are substantially reduced at term, indicating a role for NO in uterine quiescence during pregnancy and labor.

Similar changes have also been reported in the human, indicating that the NO system is a potentially important system for uterine relaxation during pregnancy.

Studies on the use of NO donors as tocolytics in the threatened preterm labor in women have been limited to very few. In 1994, transdermal patches of GTN were reported to prolong pregnancy for a mean of 59 days in a group of 13 women. In 1996, in another group of 10 women, pregnancy was prolonged for a mean of 46.2 days. The effects of GTN appeared to be due to reductions in uterine contractions in these preterm labor women. It is unknown from these studies whether NO donors are superior to other tocolytics; however, the side effects appear to be less severe.

It is unclear if the intact uterine NO system is critical for maintaining uterine quiescence during normal pregnancy. Studies in the rat and sheep indicate that inhibition of NO synthesis prior to term does not result in parturition; however, this does occur in mice. It is possible that NO may interact with other uterotonins and that the resultant effects may depend on these interactions. Moreover, uterine NO synthesis has been shown to be regulated by estradiol and progesterone, key hormones in pregnancy. Progesterone not only enhances NO synthase expression and NO synthesis in rat uterus, but also enhances NO-induced uterine relaxation. On the other hand, antiprogesterones decrease NO synthase enzymes, inhibit NO synthesis, and reduce uterine relaxation responsiveness to NO. Furthermore, inhibition of NO synthesis during pregnancy in the presence of a low dose of antiprogesterone leads to preterm labor in the rat. On the other hand, NO donors prevented prostaglandin $F_{2\alpha}$-induced preterm labor in the rat. These studies in the rat suggest that NO may interact with other uterotonins in the maintenance of uterine quiescence during pregnancy. In conclusion, uterine NO system may play a role in maintaining uterine quiescence during pregnancy, and perturbation of this system could facilitate preterm parturition.

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Appendix A

Workshop Agenda

ROLE OF ENVIRONMENTAL TOXICANTS IN PRETERM BIRTH

Sponsored by

Roundtable on Environmental Health Sciences,
Research, and Medicine
National Academy of Sciences Auditorium
2101 Constitution Avenue, N.W., Washington, D.C.
October 2–3, 2001

Workshop Goals:

- **Summarize clinical and epidemiological aspects of prematurity.**
- **Create understanding that exposures to environmental chemicals can alter gestation length.**
- **Summarize cellular, molecular, and genetic aspects of control of preterm delivery.**
- **Recognize that current *in vivo* and *in vitro* toxicological testing models are inadequately designed to capture the data that chemicals influence gestation length.**
- **Given that preterm delivery is a substantial public health concern, develop toxicological approaches to improve understanding of chemical impacts on gestational length.**
- **Use a multidisciplinary approach, including epidemiology, molecular, and so forth to better understand the mechanism underlying gestational length.**

TUESDAY, OCTOBER 2, 2001

8:30 a.m Welcome and Opening Remarks
Paul G. Rogers, J.D.
Chair, Roundtable on Environmental Health Sciences, Research, and Medicine
Partner, Hogan and Hartson

8:40 a.m. Remarks from the President of the March of Dimes
Jennifer Howse, Ph.D.

8:55 a.m. Charge to Participants and Workshop Scope
Donald Mattison, M.D.
Member, Roundtable on Environmental Health Sciences, Research, and Medicine and Medical Director, March of Dimes

SESSION I:
CLINICAL AND PUBLIC HEALTH ASPECTS OF PREMATURITY—CAUSES, INTERVENTIONS, AND CONSEQUENCES

Moderator: Jeannette Rogowski, Ph.D.
Senior Economist
RAND Graduate School

9:30 a.m. Causes and Mechanisms of Preterm Labor
James M. Roberts, M.D.
Senior Scientist and Director
Magee-Women's Research Institute

10:00 a.m. Clinical and Public Health Interventions—Why Nothing Has Worked
Robert L. Goldenberg, M.D.
Charles E. Flowers, Professor
Department of Obstetrics and Gynecology, University of Alabama at Birmingham

10:30 a.m. Long-Term Outcomes of Preterm Infants
Maureen Hack, M.D.
Professor of Pediatrics
Rainbow Babies and Children's Hospital, University Hospitals of Cleveland

11:00 a.m. Break

SESSION II:
ENVIRONMENTAL CAUSES OF PREMATURITY

Moderator: **E. Albert Reece, M.D.**
Abraham Roth Professor and Chairman
Department of Obstetrics, Gynecology, and Reproductive Sciences, Temple University School of Medicine

11:30 a.m. A Framework for Social and Cultural Determinants of Prematurity
Carol Hogue, Ph.D., M.P.H.
Terry Professor of Maternal and Child Health
Rollins School of Public Health, Emory University

12:00 p.m. Toxic Social Environment: A Factor in Preterm Birth?
Janet W. Rich-Edwards, Sc.D.
Assistant Professor
Department of Ambulatory Care and Prevention, Harvard Medical School

12:30 p.m. Lunch

SESSION III:
ENVIRONMENTAL CAUSES OF PREMATURITY: ROLE OF ENVIRONMENTAL TOXICANTS

Moderator: **Donna S. Dizon-Townson, M.D.**
Co-director
Perinatal Center, Utah Valley Regional Medical Center

1:30 p.m. Epidemiologic Clues to the Study of the Environment and Preterm Birth
David Savitz, Ph.D.
Professor and Chair in Epidemiology
School of Public Health, University of North Carolina at Chapel Hill

2:00 p.m. Exposures to Environmental Agents and Preterm Delivery
Matthew Longnecker, M.D.
Intramural Scientist
National Institute of Environmental Health Sciences (NIEHS)

2:30 p.m. Gene–Environment Interactions and Preterm Delivery
Xiaobin Wang, M.D., M.P.H., Sc.D.
Associate Professor
Boston University School of Medicine

3:00 p.m. Break

SESSION IV:
EXPERIMENTAL AND LABORATORY APPROACHES TO ANALYZING PREMATURITY

Moderator: **John R. G. Challis, Ph.D., D.Sc., FIBiol FRCOG FRSC**
Scientific Director
Canadian Institutes of Health Research, Institute of Human Development, Child and Youth Health

3:30 p.m. Current Approaches to Reproductive and Developmental Toxicity Testing and Risk Assessment
Carole Kimmel, Ph.D.
Senior Scientist
Environmental Protection Agency

4:00 p.m. Evaluating Chemical Agents for Potential Hazards in Reproduction
Jack B. Bishop, Ph.D.
Research Scientist, NIEHS

4:30 p.m. Assessment and Relevance of Environmental Chemical Effects on Uterine Muscle
Rita Loch-Caruso, Ph.D.
Professor and Director of Toxicology
University of Michigan

5:00 p.m. Reception

WEDNESDAY, OCTOBER 3, 2001

8:30 a.m. Welcome Back
Lynn Goldman, M.D.
Vice-Chair, Roundtable on Environmental Health Sciences, Research, and Medicine
Professor, Johns Hopkins University, School of Public Health

SESSION V:
BIOLOGICAL PROCESSES THAT INFLUENCE PREMATURITY

Moderator: **Lynne Wilcox, M.D.**
Director
Division of Reproductive Health, Centers for Disease Control and Prevention

8:45 a.m. Species Differentiation and Animal Models of Parturition
Peter W. Nathanielsz, Ph.D.
James Law Professor of Reproductive Physiology
Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University

9:15 a.m. Function of Steroids in Parturition and Preterm Labor
William Gibb, Ph.D.
Professor
University of Ottawa, Canada

9:45 a.m. Regulation and Assessment of Uterine Contractility and Cervical Ripening During Pregnancy
Robert E. Garfield, Ph.D.
Professor and Director
Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Texas Medical Branch

10:15 a.m. Molecular Mechanisms and Cellular Signaling Pathways Associated with Parturition
Barbara Sanborn, Ph.D.
Professor
Department of Biochemistry and Molecular Biology, University of Texas-Houston Medical School

10:45 a.m. Break

11:00 a.m. Models to Study the Actions of Uterine Lymphocytes During Pregnancy
B. Anne Croy, Ph.D.
Professor
Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

11:30 a.m. Role of Nitric Oxide in Uterine Activity and Preterm Parturition
Chandrasekhar Yallampalli, D.V.M., Ph.D.
Professor
Department of Obstetrics and Gynecology, University of Texas Medical Branch-Galveston

12:00 p.m. Fetal Size and Preterm Birth
Stephen J. Lye, Ph.D.
Professor and Joint Head
Program in Development and Fetal Health, Samuel Lunenfeld Research Institute, Mount Sinai Hospital Departments of Obstetrics and Gynecology and Physiology, University of Toronto

12:30 p.m. Lunch

SESSION V:
DISCUSSION

1:30 p.m. Summation of the Workshop
Donald Mattison, M.D.
Member, Roundtable on Environmental Health Sciences, Research, and Medicine
Medical Director, March of Dimes

2:00 p.m. Panel Discussion

Woodie Kessel, M.D., M.P.H.
Assistant Surgeon General
U.S. Department of Health and Human Services

Mark Klebanoff, M.D.
Director
Division of Epidemiology, Statistics and Prevention Research, National Institute of Child Health and Human Development

Allen J. Wilcox, M.D., Ph.D.
Chief of Epidemiology Branch
Environmental Diseases and Medicine Program, NIEHS, Division of Intramural Research

Daniel Krewski, Ph.D.
Professor and Director
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Fernando Guerra, M.D.
Director of Health
San Antonio Metropolitan Health District, Texas

Catherine Spong, M.D.
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4:30 p.m. Adjournment

Appendix B

Speakers and Panelists

Jack Bishop, Ph.D.
Director
National Institute of Environmental Health Sciences
National Institutes of Health, Toxicology Branch

B. Ann Croy, Ph.D.
Professor
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University of Texas Medical Branch

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Lynn R. Goldman, M.D.
Professor
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Johns Hopkins University School of Public Health

Fernando Guerra, M.D.
Director of Health
San Antonio Metropolitan Health District

Maureen Hack, M.D.
Professor of Pediatrics
Rainbow Babies and Children's Hospital
University Hospitals of Cleveland

Carol Hogue, Ph.D., M.P.H.
Director and Jules and Deen Terry Professor of Maternal and Child Health
Rollins School of Public Health
Emory University

Jennifer Howse, Ph.D.
President
March of Dimes

Woodie Kessel, M.D., M.P.H.
Senior Child Health Science Advisor
U.S. Department of Health and Human Services

Carole Kimmel, Ph.D.
U.S. Environmental Protection Agency Headquarters

Mark Klebanoff, M.D.
Director
National Institute of Child Health and Human Development

Daniel Krewski, Ph.D.
Department of Epidemiology and Community Medicine
University of Ottawa

Rita Loch-Caruso, Ph.D.
School of Public Health
University of Michigan

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Intramural Scientist, Epidemiology
National Institute of Environmental Health Sciences

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Mount Sinai Hospital

Donald Mattison, M.D.
Medical Director
March of Dimes

Peter W. Nathanielsz, Ph.D.
James Law Professor of Reproductive Physiology
Department of Biomedical Sciences
Cornell University College of Veterinary Medicine

Albert Reece, M.D.
Abraham Roth Professor and Chairman Temple University School of Medicine
Department of Obstetrics and Gynecology
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Janet Rich-Edwards, Sc.D.
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Harvard Medical School

James M. Roberts, M.D.
Senior Scientist and Director
Magee-Women's Research Institute

Paul Rogers, J.D.
Partner
Hogan & Hartson

Jeannette Rogowski, Ph.D.
Senior Economist
The RAND Corporation

Barbara Sanborn, Ph.D.
Professor
Department of Biochemistry and Molecular Biology
University of Texas-Houston Medical School

David Savitz, Ph.D.
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Catherine Spong, M.D.
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National Institute of Child Health and Human Development
National Institutes of Health

Xiaobin Wang, M.D., M.P.H., Sc.D.
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School of Medicine
Boston University

Allen Wilcox, M.D., Ph.D.
Chief, Epidemiology Branch
National Institute of Environmental Health Sciences
National Institutes of Health

Lynne Wilcox, M.D.
Division Director
Centers for Disease Control and Prevention
National Center for Chronic Disease Prevention and Health Promotion

Chandrasekhar Yallampalli, D.V.M., Ph.D.
Professor
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University of Texas Medical Branch

Appendix C

Workshop Participants

Carolyn Alexander
Momease/March of Dimes, Professional Services Committee Member

Marilee Allen
Johns Hopkins University School of Medicine

Phillip Archer
Virginia Union University

Achilles Athanassiou
New England Medical Center/Tufts University

Marta Baez
New York State Department of Health

Susan Bakewell-Sachs
The College of New Jersey

Susanne Bathgate
George Washington University

Janis Biermann
March of Dimes

Amy Branum
Center for Disease Control, National Center for Health Statistics

Julie Broussard
Louisiana State University, Health Sciences Center—Earl K. Long Medical Center

Susan Brunssen
National Institute of Environmental Health Services, National Institute of Health, and the University of North Carolina at Chapel Hill

Germaine Buck
National Institute of Child Health and Human Development

Barbara Caldwell
University of Medicine and Dentistry of New Jersey School of Nursing

Quintin Clark
Sarasota County Health Department

Betty Connal
Inova Fairfax Hospital

Cynthia Conventon
Pennsylvania Department of Health

Deborah Cordrey
Montgomery County Health and Human Services

Barbara Coyle
Division of Public Health, Bureau of Occupational Health, Wisconsin Department of Health and Family Services

Susan Cummins
National Center for Environmental Health, Centers for Disease Control and Prevention

Shelley Davis
Farmworker Justice Fund

Dona Dei
National Capital Area Chapter March of Dimes

Todd Dezen
March of Dimes

Anna Dillingham
Health Track

Sean Donohue
Senate Committee on the Environment and Public Works, United States Senate

Maureen Edwards
Maryland Department of Health and Mental Hygiene

Sara Elias
University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School

John F. Evans
Kansas Department of Health and Environment

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Elaine Francis
United States Environmental Protection Agency

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Porter Novelli

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Christina Manero
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Claudia Miller

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United States Environmental Protection Agency

Richard Miller

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Coventry Health Care

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Center for the Evaluation of Risks to Human Reproduction

Michael Narotsky
United States Environmental Protection Agency

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Japanese External Trade Organization, New York

Mary Ellen O'Connell

Gilbert Omenn
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Centers for Disease Control, National Center for Health Statistics

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Mid-Atlantic Center for Children's Health and the Environment

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School of Rural Public Health

Joann Petrini
March of Dimes

Pat Phibbs
Bureau of National Affairs Chemical Regulation Reporter

Aron Primack
National Institutes of Health, Fogarty International Center

Alan Roberson

Al Rosenfeld
March of Dimes

Beth Rowan
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Farmworker Justice Fund

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Offie Soldin
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Emy Lou Solomon
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Ann Umemoto
March of Dimes

Steve Via
American Water Works Association

Donna Vivio
American College of Nurse–Midwives

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New Jersey Medical School of Women's Health

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George Washington University, Biostatistics Center

Keith Zachman
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Jim Zhang

Diana Zuckerman
National Center for Policy Research for Women and Families