Schizophrenia and related disorders are adult-onset illnesses with no definitively established risk factors. Several studies report that exposures to infection and nutritional deprivation during early development may elevate the risk of later developing schizophrenia, specifically during the prenatal period. Preliminary evidence implicates lead exposure as well, suggesting that chemical exposures during early development may constitute a new class of risk factors for schizophrenia that has not been adequately investigated. Exposure to lead is given as an example of a chemical agent for which some effects have been described throughout the life course on both general neurodevelopmental outcomes and now on a specific psychiatric diagnosis. Findings from prospectively collected birth cohorts are offered as examples of both innovations in methodology and opportunities for future generations of investigators. Key words: developmental, lead, Pb, prenatal, prospective, psychosis, schizophrenia.

The Prenatal Determinants of Schizophrenia (PDS) study was a collaborative, prospective cohort study of approximately 20,000 pregnant women enrolled in northern California between 1959 and 1966 as part of the Childhood Health and Development Study (Susser et al. 1959 and 1966 as part of the Childhood Health and Development Study (Susser et al. 1959 and 1966). They have been used in combination with hospital records and new diagnostic data (van den Berg et al. 1988).

Cases of schizophrenia and SSDs were identified from a database of inpatient, adulthood, decades after the putative prenatal exposures. Systems to track subjects, identify exposures, and diagnose disease must be maintained for decades. In addition, large numbers of subjects are required to accurately assess the relatively modest increases in risk that any single factor is likely to contribute to a multifactorial disease such as schizophrenia.

Recent investigations have built on these studies, using prospective cohorts identified before birth for studies of known or suspected neurodevelopmental disruptors. Several ascertain prenatal exposure through quantifiable measurements, for example, analysis of archived maternal biologic samples collected before birth. Various hypotheses have been advanced, and a number of studies have produced suggestive results. As an example, we describe one ongoing study that has examined toxic, nutritional, infectious, and other risk factors. After describing selected findings on infection and nutrition that illustrate the methods used, we then describe how this study has been used to investigate prenatal lead exposure as a risk factor.

The PDS study. The Prenatal Determinants of Schizophrenia (PDS) study was initiated in the 1990s. It is based on a cohort of approximately 20,000 pregnant women identified in northern California between 1959 and 1966 as part of the Childhood Health and Development Study (Susser et al. 2000). This study includes aliquots of maternal sera drawn during prenatal visits. These samples were stored and maintained at National Institutes of Health facilities, frozen at −20°C in anticipation of future studies. They have been used in combination with hospital records and new diagnostic data (van den Berg et al. 1988).
subjects in this study had single samples taken
antibody titers taken in serial samples. As most
conversion is characterized as a 4-fold rise in
H2N2/Japan/62, A/H2N2/Taiwan/64, and
California, including A/H2N2/Japan/57, A/
gens of influenza strains known to be preva-
inhibition test was performed on four anti-
and matched controls, the hemagglutination
analysis in stored maternal serum from cases
studies capable of performing serologic
1973). The PDS study is among the first
agents has been considered for some time and
available serum samples (for details see Susser
maternal blood draw, and the number of
factors, including date of membership in the
cohort, date of birth, gender, timing of first
maternal blood draw, and the number of

Influenza and markers of infection.
Previous work describing associations
between prenatal exposure to a variety of viral
agents has been considered for some time and
extensively reviewed elsewhere (Crow 1978;
Mednick et al. 1988; Torrey and Peterson
1973). The PDS study is among the first
studies capable of performing serologic
measures for exposure to influenza. For this
analysis in stored maternal serum from cases and
matched controls, the hemagglutination
inhibition test was performed on four anti-
gens of influenza strains known to be preval-
ent between 1959 and 1966 in northern
California, including A/H2N2/Japan/57, A/
H2N2/Japan/62, A/H2N2/Taiwan/64, and
B/Massachusetts/66. Exposure to influenza
usually results in a rise in antibody titers,
referred to as seroconversion. Typically, sero-
conversion is characterized as a 4-fold rise in
antibody titers taken in serial samples. As most
subjects in this study had single samples taken
within each trimester, a single cutoff level was
sought as a proxy of influenza exposure during
pregnancy. Validity studies demonstrated that
levels of ≥ 1:20 in a single serum sample were
highly specific and sensitive.

First-trimester exposure was associated a
7-fold increase in risk of schizophrenia and
SSDs, whereas second- and third-trimester
exposure showed no increase in risk. However,
although first trimester is usually defined as the
period between zero and 90 days after the
last menstrual period (post-LMP), the blood
draws taken in this study only occur as early as
46 days post-LMP. Therefore, first trimester
does not necessarily signify, in effect, assessment in the latter
part of first trimester. Additional analyses were
conducted analyzing exposure during the first
and second halves of pregnancy defined as
0–142 days (in effect, 40–142 days post-
LMP) and from 143 days post-LMP until ter-
mination of pregnancy, respectively. Exposure
in the first half of pregnancy conferred a
3-fold increase in risk, whereas no increase was
seen after exposure during the second half
of pregnancy or when second-trimester exposure
was considered.

Although clearly an advance over previous
work, the PDS study has three key limitations.
First, the number of cases of schizophrenia and
SSDs with the required prenatal sera was small—64 cases and two matched controls per
case. Although the study found a substantial
association between prenatal influenza expo-
sure and schizophrenia, the confidence limits
of this association are wide. Second, influenza
infection is typically documented by noting an
increase in titers over time, and the measure
used in this study represents a proxy of the
established standard. Third, the increase in
risk does not correspond exactly with previous
findings concerning timing of exposure. Prior
reports have indicated that second-trimester
exposure is associated with increases in risk,
whereas in this study, exposure during first
trimester and first half of pregnancy confers
risk. Further investigation is required to
explain this difference.

Prenatal maternal nutrition and body
mass index. Nutritional factors has also been
postulated to play a role in the etiology of
schizophrenia. Both lack of specific micronu-
rients and general nutritional deprivation have been previously implicated as risk fac-
tors for broad developmental disruption and
for schizophrenia specifically. In one land-
mark study of prenatal nutritional depriva-
tion known as the Dutch Famine Study
(Susser et al. 1998), neurodevelopmental
outcomes were measured after severe caloric
restriction. Rates of schizophrenia approxi-
mately doubled for individuals conceived under
conditions of nutrient deprivation
during early gestation (Susser et al. 1996).

Early gestational exposure to famine con-
ferred risk for schizophrenia, whereas late
gestational exposure did not. Later studies
that extended these findings to schizophrenia
spectrum personality disorders also showed a
2-fold increase in risk for early gestational
exposure to famine (Hoek et al. 1998). Two
other studies found evidence that low mater-

nial body mass index (BMI) or low birth
weight is associated with schizophrenia
(Done et al. 1991; Wahlbeck et al. 2001).

Recently, high rather than low maternal
BMI has become a focus of concern because
the number of women of reproductive age
with above-average or high BMI has increased
in industrialized societies. The PDS study
used measures of prepregnant maternal
BMI, categorized to low (< 19.9), average
(20–26.9), above average (27–29.9), and high
(≥ 30.0). Compared with average maternal
prepregnant BMI, high BMI was significantly
associated with schizophrenia and SSDs in
the adult offspring (relative risk = 2.9; 95%
confidence interval, 1.3–6.6). This finding
was independent of maternal age, parity,
race, education, or cigarette smoking during
pregnancy.

Prenatal Lead Exposure and
Neurodevelopment
For centuries lead has been known as a toxic
agent but only recently has been recognized as
having subtle but significant developmental
effects. McKhann stated in 1926 that the
“manifestations of Pb poisoning usually sub-
side without serious consequences.” In 1943
Byers and Lord (1943) disproved this state-
ment in a follow-up study of 20 children with
documented Pb poisoning. They examined
not only gross neurologic signs but also IQ
scores and academic performance. Although
based on a small sample of convenience, 19 of
the children later exhibited serious difficulties
in school. Since these initial studies, prenatal
Pb exposure has been measured using mater-
nal blood Pb (BPb) during pregnancy, neo-
tal BPb, amniotic fluid, and umbilical cord
PBp (Korpela et al. 1986). Comparisons of
maternal and umbilical BPb indicate that
transfer of Pb from maternal to fetal blood
during pregnancy is unimpeded by the pla-
enta. Prospective approaches to Pb exposure
and development have been used in a number
of instances. They have focused primarily on
developmental outcomes such as attention,
academic achievement, and cognition, and
have used maternal blood draws or postnatal
measures in a variety of biologic media
(Pocock et al. 1994). These studies and others
generally have provided strong support for the
role of Pb as a developmental neurotoxin
(Bellinger et al. 1994). However, because they
mostly have followed subjects into or through
childhood, they are not informative regarding
adult-onset disorders such as schizophrenia.

A few modestly sized studies have now
followed subjects through adolescence. In one
example, Needleman and colleagues recruited
312 first- and second-grade children in
Chelsea and Somerville, Massachusetts.
Dentin Pb levels were measured for each sub-
ject (Needleman et al. 1979). This measure
was used to identify high exposure (those with
dentin levels > 20 ppm), moderate exposure
(10–19.9 ppm), and low exposure (< 10 ppm)
(Needleman et al. 1990). Neurobehavioral
testing was conducted at the time of collect-
ion in 1979 (mean age, 7.3 years) and again
in 1988 (mean age, 18.4 years). Dentin Pb is
useful as a measure of exposure averaged over
the age of the tooth, although dentin Pb
levels are associated with dental caries and
fillings (Gil et al. 1996). Results showed an
increased risk of not graduating from high
school among those with increased dentin Pb
levels. Reading difficulties sufficiently severe
to be defined as a disability showed a similar
distribution. Subjects who had been diag-
nosed with clinical Pb poisoning earlier in
the study had the highest percentages of
failure to graduate (42.9%) and reading
disabilities (50%).

Opler and Susser
A study by Dietrich et al. (2001) presents data that show Pb exposure versus juvenile delinquency at different exposure levels. Based in Cincinnati, Ohio, the sample of 195 subjects is largely African American, disadvantaged, and urban. Using a prospective cohort with prenatal and postnatal Pb assessments collected every 3 months until 6.5 years, the study measures parental and self-report of delinquent behaviors including drug and alcohol use in adolescence. Subjects were given self-report questionnaires and assessed at 15 and 17 years of age. The results are categorized by lowest, low, medium, and highest Pb levels. When prenatal Pb, average childhood Pb, and 78-month Pb were estimated as predictors of delinquent behavior, increasing concentrations were associated with a modest increase in delinquent acts reported in adolescence. Prenatal Pb exposure > 10 µg/dL results in an increase of more than 2.3 delinquent acts compared with exposures ≤ 5 µg/dL. Significantly higher rates of delinquent behavior are related via a categorical Pb measured prenatally and at 78 months of age, although not by average childhood Pb (Dietrich et al. 2001).

Findings on Prenatal Lead Exposure and Schizophrenia

Many of the effects described in adolescence after early-life exposure, including decreased academic attainment, social deficits, and behavioral difficulties are comparable with the early antecedents of SSDs. Similarly, a number of factors that have been suggested as being associated with Pb exposure, such as urban residence, have also been studied as risk factors for SSDs. Although the samples from prospective studies described here do not have sufficient power to be definitive, the findings are suggestive, and the overall approach that these studies take may be used as a model. Principally, the combination of prospective collection of biologic samples can be combined with longitudinal assessments for the study of early-life exposures as they relate to adolescent and adult psychiatric diseases.

Although several techniques are available for assessing Pb exposure in biologic samples, the principal one used in studies of prenatal exposure is direct measurements on maternal blood. The PDS study has stored sera, not whole blood, containing the Pb-sequestering erythrocytes required for direct measurements in small volumes. Techniques for direct measurement of Pb could not be employed. However, a biologic marker of Pb exposure, δ-aminolevulinic acid (δ-ALA) may be detected in urine, plasma, and serum using high-pressure liquid chromatography with fluorescence detection.

Feasibility studies were conducted to assess the utility and predictive value of this technique in small volumes of stored maternal serum (Opler et al. 2004). It was determined that second-trimester serum was likely to be the best indicator of prenatal exposure because both Pb and corresponding δ-ALA levels are believed to be relatively stable at midpregnancy. Second-trimester samples were available for 44 cases and 75 matched controls (one to two controls per case).

A single 100-µL aliquot of second trimester serum was made available for each subject. A concentration of 9.5 ng/mL of δ-ALA, corresponding to a Pb level of 15 µg/dL, was used as a cut-off value to divide the sample into exposed and unexposed subjects. Samples were coded and blinded with respect to case status. Using this approach, Pb exposure as measured by elevated δ-ALA was associated with about a 2-fold increase in risk of SSDs in this sample (odds ratio = 2.43; 95% confidence interval, 0.99–5.96). The small numbers of subjects contribute to the wide confidence limits.

Some important limitations should be noted. First, the use of a biologic marker rather than direct measurements means that the observed increase in risk could be mediated by the effects of Pb on δ-ALA rather than by Pb exposure directly. Serum δ-ALA itself may be the exposure of interest. In experimental models, δ-ALA has been shown to be neurotoxic, interfering with GABA (γ-aminobutyric acid) neurotransmission [Emanuelli et al. 2001; also reviewed by Cory-Slechta (1995)]. Second, the findings of this study are also difficult to interpret conclusively because the sample size is relatively small and the result has a wide confidence interval. We have now obtained permission to analyze the one other existing data set of this type with a similar sample size. These results will be forthcoming.

Future Directions

The use of prospectively collected cohorts in combination with archived biologic samples is a proven and powerful method for studying disease–exposure relationships throughout the life course. This method has allowed schizophrenia research to move away from less refined definitions of prenatal exposure and into investigations that may someday focus on specific molecular agents in causal pathways. This longitudinal approach was made possible by the foresight of early generations of researchers in combination with the efforts of those who succeeded them. The initial results from prenatal cohort studies are still preliminary, and the process they describe is still in its infancy. Every class of candidate exposures will benefit from continued technical and methodologic refinement. Using infection as an example, those agents or strains that cause the greatest increases in risk, specific physiologic responses to infection, and the timing of exposure during pregnancy may be further investigated. Nutritional deprivation might also be explored in greater detail, using methods to study the roles of individual micronutrients. Finally, chemical exposures could eventually be examined in terms of toxicokinetics and mechanisms of action, allowing proximal effects of exposure to be teased apart and considered separately from consequent physiologic responses.

Although life-course epidemiology is currently yielding important results, it is limited to the resolution and specificity that designers of prenatal cohorts create through the type, frequency, and periodicity of data collection. Presently, the biology that links exposures to causal mechanisms is nearly impossible to study in detail without the use of experimental models. Basic researchers have used clinical and neurochemical observations from humans to develop animal models of schizophrenia and are now studying some of the exposures implicated in epidemiology using the same techniques. Although animal models of psychiatric disorders are imperfect and subject to a number of limitations, they are useful for testing the biologic plausibility of new hypotheses generated by epidemiology.

We believe that to reach the goal of effective prevention of schizophrenia, all available data on the disorder must be integrated, including observational and experimental findings. Investigators with interdisciplinary training and who are comfortable with the language and concepts of study design from the population level to the molecular level will play a crucial role in the future of the field.

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