SPECIAL ARTICLE

Laboratory Research on Problems of Fetal and Early Life*

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In opening a symposium in the city of Philadelphia on problems of fetal and early life, I am reminded of Benjamin Franklin's reply to a question about why he experimented with electricity. His answer can be the rhetorical theme of this symposium: "What good is a new-born baby?"

Healthy babies are a social asset and can predictably develop into healthy adults. Ill or ill-formed children are an emotional and financial burden to society; additionally, abnormalities of the early developmental period can be specific risk factors for disease in later, adult life. Research designed to improve the chances of a healthy developmental period for genetically sound babies is a social and medical imperative. Past and current laboratory contributions to research on the problems of fetal and early life are amply illustrated in the applications described in the symposium presentations that follow. Let us concentrate on a group of unresolved issues and some of the approaches to their solution that are suggested by our present knowledge, if not always by our level of technologic development. Where feasible, laboratory approaches to problems based upon recent advances in our knowledge will be selectively described.

There are two, at least two, interdependent forces that determine the nature of the free-living product of conception: first, its genetic constitution, and secondly, the constraints imposed by the process of development that determine what is structurally feasible. This view derives from current studies in evolution that try to explain why there are gaps in morphologic space (e.g., why there are no animals with wheels instead of legs, nor any covered by a cross between feathers and hair). Igor Dawid puts it thus: "Even if, for instance, we knew the total sequence of the human genome, we wouldn't know what a human being looked like." The point of raising what is essentially an evolutionary concept is to emphasize the need to look at the developmental process, and damage to it, independently of the genomic determinants of human form and function. Small modifications, for example, of the timing of the developmental process may cause fetal loss, or may produce disproportionately large changes in the morphology and function of the adult.
Having offered these principles as a basis for examining research in genetics and developmental medicine, specific instances that require attention shall be noted. The most direct approach is to define the “high risk” infant, the child most likely to succumb to disease or death, and ask how he got that way.

Infant mortality (under one year of life) in the United States in 1980 was estimated at 1,251.4 for every 100,000 live births. Under 28 days of age, about 840 died for every 100,000 live births.

Of all deaths under one year of age, the statistically leading categories expressed as rates per 100,000 live births are shown in table I.

As an additional background datum, low birth weight babies (less than 2500 grams), a group recognized to be a high-risk population, made up 6.9 percent of all live births in 1979. The greatest numbers were found among black infants (12.6 percent compared to 5.8 percent among whites), among young teen-age mothers (14.5 percent among mothers under 15 years of age), and among mothers in their forties.13

To these figures must be added an estimate of fetal loss at a significant multiple of the number of live births; the magnitude of the question, “Why?” becomes overwhelming.

To facilitate a presentation of the problems, their possible solutions, and the laboratory’s role in their investigation, this manuscript shall be confined to the two major sets of factors that influence the successful completion of development: the genetic constitution and the developmental process, each of which can go awry to adversely affect the outcome.

**Genetic Factors: The Abnormal Genome**

Abnormalities of number and types of chromosomes are very gross genetic changes that can be associated with some infant disorders, e.g., trisomy 21 in Down’s syndrome, although the observation of association tells us nothing about how the disorder and the abnormality are related. The frequency of chromosomal abnormalities is described in a study3 of 54,746 newborn babies. Sex chromosome abnormalities were found in 1:400 males and in 1:700 females; autosomal trisomies in 1:800 births; and overall...
fragments in abnormal locations, such as micronuclei in maturing erythrocytes, provides yet another type of chromosome analytic tool that has proven useful in the study of some forms of metal-associated carcinogenesis and may become useful in perinatal analysis.

All of these techniques, though readily applicable in clinical practice as monitoring systems, and useful in statistical analyses for risk prediction, support the relationship of the observation and the disorder but do not really get at the problem. Every time such relatively gross chromosomal changes are seen, it is assumed that there are single gene changes at the molecular level (point mutations), or alterations of the linear coherence of DNA strands, or disturbance of the segregation of chromosomal DNA through abnormalities of the spindle fiber mechanism in nuclear division. Changes at these levels of discrimination must await appropriate technologies. Eventually, it is our hope that base-sequence changes in DNA can be related to abnormalities of form and function.

Even then, the issue we are trying to resolve will remain unanswered: how does a specific change in DNA become expressed as an abnormality? The characterization of the sequence of bases in human DNA is within sight now that restriction endonucleases can be applied to DNA preparations and base sequencing is becoming a relatively common research procedure. The discovery of interrupted functional base sequences, of introns and exons, and the complex systems for stitching together separated base sequences to produce functional templates only makes the problem of relating abnormalities to DNA changes a bit more complex than anticipated.

The next, and at the moment most difficult, step will be to relate DNA sequence variants with normal and abnormal cell products, and from there to trace the chain of biochemical and biophysical events to their final expression as clinical disease or abnormality. That the relationship exists and that understanding it is an attainable objective can be seen in the development of our understanding of the molecular biology of sickle cell anemia.

Studies of the transmission of mutations are more difficult. Chromosomal mutations inherited in the germinal cell line have been related to 30 percent to 50 percent of spontaneous abortions and to 0.5 percent to 0.7 percent of live births with chromosomal abnormalities.19 However, there is no evidence indicating that there is an increase in the incidence of chronic and genetic abnormalities among the offspring of a human population exposed to a chromosome-breaking agent. Schull, Otake, and Neel16 analyzed children of the atomic bomb survivors conceived after parental exposure to ionizing radiation. They failed to show any statistically significant effect of the parental exposure in the F1 generation. Their work illustrates the range of studies needed to study this type of analysis and the place of the laboratory among them.

Clinical examination and autopsy studies include "untoward outcomes," including major congenital defects, stillbirth, and death during the first week of life; survival of liveborn infants; whereas laboratory research includes the study of chromosomal abnormalities and alterations of an array of specific proteins by analysis of protein phenotypes.

On the other hand, exposures of fetuses to radiation in utero, results in significant clinical effects as described for the Japanese atomic bomb population (mainly microcephaly)11 and for diagnostically irradiated fetuses, as described by Russell and Russell.15 At the opposite end of our analytic capacities lies our ability to recognize and measure gene products without yet being able to associate them with gene sequence changes. The laboratory technology for estimation of Factor VIII and Factor IX is
available and precise enough to monitor the risks of hemophilia and von Willebrand’s disease. Similarly, the severity of β-thalassemia can be predicted by analyses of β-thalassemia hemoglobin variants. In such instances as these, the knowledge and technology is adequate for diagnosis and prediction but is insufficient for an understanding of the development of variants or of the gene determinants of disease. Final success will be an understanding of the entire sequence of cause and effects and the ability to place corrections or modulations into the system to prevent the clinical disease.

Yet another significant laboratory advance can be expected shortly. It will be possible to expand the monitoring of newborns for abnormalities that would not be expressed until later life, thereby making it possible to avoid some of them. As an example, persons with chemically inducible lupus erythematosus after exposure to hydrazines or isoniazid are genetically slow acetylators, the ultimate culprit in the system probably being an unacetylated aromatic amine group affecting the immune system. It is inevitable that screening will be developed for slow acetylators and avoidance of exposure for those at risk. It also seems that idiopathic lupus erythematosus patients are also slow acetylators, leading to an even more significant role for screening. Very little is known about the development of the liver’s acetylation capacity in the fetus and newborn, information that is essential if deviations from normal are to be recognized, nor is it known how the genetic control of acetylation is exercised, information that would be useful if corrective measures were to be taken.

Better knowledge of the heritable genomic variations that affect fetal and infant survival and health, even without an understanding of how the genome produces the disorder, would be of value only for monitoring purposes were it not for the possibility of correcting the system. Correction of genetic abnormalities would be the ultimate success, either by molecular surgery on the gene or by supplementation of the affected cell product. The prospects for these ends have improved with the development of recombinant DNA technology but must await more detailed knowledge of the significant chromosomal lesions and their specific effects.

The Developmental Process

The second major factor in the production of healthy babies is the developmental process. This process begins with the fertilized ovum that has acquired its genome and progresses through intrauterine life and extrauterine development to the postpubertal age. For purposes of this discussion, these comments will be restricted to intrauterine development and postnatal life to one year of age.

The first and most critical of all research efforts is to develop a better and more detailed understanding of the normal developmental process. At the same time, the mechanisms must be defined whereby normal development is perturbed resulting in failed or poor products of conception. The latter is perhaps the easier of the two research foci, and much has already been achieved in that area.

The laboratory is the only place that the questions pertaining to the normal developmental process can be addressed. There is an awareness of many varieties of maturation processes that develop at different stages of organismal development. These include the development of normal bilirubin metabolism, the induction of normal enzyme activities, and shifts of cell distribution during the development of competent hematopoiesis, the orderly development of cellular and humoral immune mechanisms, and the classical anatomic-embryologic analyses of the development and organization of the whole human organism. A vast body of essential knowledge is yet to be uncovered about
normal development: the chemical and physical features that are the basis for cell and tissue development, the organization of tissues, and the means whereby tissue activities are related into a coherent whole. Our current information has led to concepts of time-related development wherein shifts of time-frame of one developmental feature may place the entire organism in jeopardy. That is one way that one could view a delay in neural tube closure at different times in development leading to major or minor spinal cord and vertebral anomalies. A comparable time frame study demonstrated that minor elevations of core body temperature of pregnant bonnet monkeys on specific gestational days caused fetal resorptions and fetal malformations, indicating the existence of a teratogenic-sensitive period for temperature changes. It is necessary to use the laboratory when clinical opportunities arise to understand such processes as the development of the capacity to acetylate compounds, to activate superoxide dismutases and dehydrogenases, to permit or to block adduct formation with nucleic acids, in short to describe the orderly and infinitely interlocked maturational development of the human organism.

Turning to a much more familiar field, but one beset by a multiplicity of factors, the study of perturbations of the developmental process is at once an observational, a biostatistical and, finally, a laboratory research effort. Given the universe of the potential means of adversely affecting the developmental process, selection of a target for study is crucial to effective and useful research. For this, the sensitive clinician and the aware scientist must come together to create the necessary hypotheses. The recognition of an association of maternal x-irradiation with fetal damage in utero is such a construct. So too would be hypothesis of fetal loss owing to exposure to an environmental chemical agent.

After the hypothesis has been stated, the most useful initial tool for investigation is an epidemiologic study that can test the degree of assurance that the association is truly a related set. Unfortunately, epidemiologic analysis is a very coarse tool that will fail when numbers are too small, when background "noise" is too great, or when endpoints are ill-defined. A startling example of the endpoint problem in epidemiology is the various practices of defining prematurity and low birth weight throughout the world. Should one wish to try to associate prematurity or low birth weight with some factor, say the nutritional status of the mother, one would not be able to do so using worldwide data because different societies and different countries define the two terms differently. Here the clinical scientist and the laboratory can help to define the parameters to be studied and characterize the reliability of the endpoints under study.

The next step in the investigation is the selection of the targets of study in the laboratory. In these days of environmental consciousness, if not conscience, the clinical laboratory must be prepared to study, qualitatively and quantitatively, a variety of prospective injurious agents. Not only must the presence of an agent be shown—or the presence of a product of the agent—but the exposure must be quantified. The quantitation is particularly important since dose-response relationships will be needed to supply critical proof of the hypothetical association. Some of the technologies to identify and measure materials that could perturb the developmental process exist in various kinds of laboratories, clinical, toxicological, and industrial. Some will have to be invented.

A useful classification of the perturbing mechanisms and the laboratory contribution to their understanding is division into physiological groups: those principally affecting metabolic development, those affecting immunologic and hematologic development, those affecting normal structural organization, and those affecting cell-to-cell and tissue-to-tissue communication.
Metabolic development can be a target for modification by exogenous chemical agents. The recent evidence that alcoholics have impaired ability to metabolize acetaldehyde owing to a deficiency of a soluble fraction of acetaldehyde dehydrogenase suggests several leads to the study of the "fetal alcohol syndrome." The unmetabolized acetaldehyde is claimed to have a morphine-like effect in the central nervous system of the alcoholic and can cross the placenta to affect fetal neurological development. The rate of the development of the aldehyde dehydrogenase system in the fetus should be examined to determine whether it, too, is impaired by an inherited defect or by toxic depression.

Another metabolic effect, thus far well demonstrated in rats, is that prenatal exposure to a common antidepressant drug, imipramine, results in behavioral and neurochemical effects that last well beyond the period of drug exposure. Not only are exposed rat pups irritable, with poor temperature regulation and decreased muscle tone, but brain dopamine levels were altered as were the number of beta-adrenergic receptors. This can be interpreted as an example of drug-induced developmental perturbation of metabolic systems.

Metabolic development is also influenced by endogenous materials. Delayed release of gonadotropic hormones or interference with the ovarian secretion of estrogen can produce defective oocytes. Such changes of endocrine function can be induced, in turn, by exogenous agents, including exogenous hormones.

The list of environmental agents that can affect the development of the conceptus, probably through metabolic alterations, is long and includes gases (such as ozone, carbon monoxide, and anesthetics), metals (lead, mercury, cadmium, selenium), drugs and hormones (e.g., thalidomide, diethylstilbestrol, alcohol), and organic pesticides, herbicides and solvents (e.g., dioxins, polychlorinated biphenyls, 2,4-D, 2,4,5-T, benzene, toluene). The laboratory must be prepared to monitor such agents carefully to relate exposures to clinical consequences. Furthermore, laboratory investigators must develop the insight and the techniques to characterize the specific effects of such exposures.

Laboratory study of immunologic and hematologic development has become highly sophisticated and is widely applied. Questions of heme and globin synthesis, timed development of hemoglobin types, evolution of the immunoglobulin species, and the development of lymphocyte populations (including T, B, and various subgroups) are well on the way to resolution. However, interference with normal immune and blood development is not well understood. The reason for anemia or immunoincompetence in babies is not always clear. Testable hypotheses are needed for these calamities. For example, one explanation for immunologic incompetence came from observations on malnourished children with a major defect in cellular immunity—one of the features of acrodermatitis enteropathica. This immunologic failure is responsive to zinc supplementation, and the recognition of the deficiency is highly dependent upon accurate and standardized laboratory analytic facilities.1 Laboratory science should be used not only to monitor the abnormalities but also to explain the mechanism and, eventually, define the cause.

Perturbation of normal structural development is much more difficult to explain. A vast amount of effort has gone into descriptions of malformations and attempts to relate them to embryologic development. Some of these studies are detailed enough and of sufficiently narrow scope to be useful. Others are still poorly defined. Laboratory science has provided means of monitoring the development of the fetus and predicting certain abnormal structural events. For example, neural tube defects afflict some 6,000 newborn infants per year in the United States. It is assumed that genetic and environmental
factors play a role in their development. Without enough insight to offer specific hypotheses regarding cause or mechanism, the laboratory has provided a means of antenatal monitoring of hydrocephalus and spina bifida with radioimmunoassay of \( \alpha \)-fetoprotein in maternal serum, combined with intrauterine sonography, x-ray, and fetoscopy.\(^5\)\(^-\)\(^10\)

Disturbances of structural development must eventually be understood in biochemical and biophysical terms. The breakthroughs in this obdurate field will probably come from fortunate observations of association between chemical or physical causative agents and relatively minor structural aberrations that will allow for survival. The scientist with an interest in this area will have to be particularly aware of clinical opportunities and will need all the technologic resources of the laboratory to test his ideas.

One of the most exciting fields of biological research deals with communication and control among cells and tissues. Scientists have long been accustomed to dealing with hormonal messengers and synaptic junction messengers and are becoming familiar with the sequence of messengers that permit transfer of specific stimuli across cell membranes into the intracellular and intranuclear environments. The development of these systems is imperfectly understood. To take but one example, cells derived from normal mature organisms and grown in culture display the phenomenon of contact inhibiton—the cessation of cell division when cells are in physical contact with one another. The same phenomenon is seen in epithelial regeneration in wound healing. Yet in the developing embryo and fetus, contact inhibition seems to be absent and, as maturation occurs, the phenomenon is displayed in different tissues at different times.

The development of the control systems for orderly cell proliferation is subject to interference during development, the most obvious examples being the induction of teratologic abnormalities. Rather than belabor the story of thalidomide once more, permit an illustration of teratologic perturbation by returning to the fascinating tale of zinc deficiency. Low concentrations of zinc in maternal serum are related to abnormal labor, prematurity and immaturity, and congenital malformations in man.\(^7\) In animals, zinc deficiency is likewise a teratogen, as well as a cause of decreased pulmonary surfactant phospholipids and decrease in zinc-containing enzymes such as chymotrypsin A.\(^6\) Here, then, is a developmental alteration, frequently appearing as a malformation, associated with a deficiency of a metal essential for over 120 metalloenzymes (e.g., carbonic anhydrase, carboxypeptidase A).

These enzymes play a role in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids, and they encompass all known types of enzyme activity: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. They participate in the stabilization of ribonucleic acid (RNA) and DNA and are components of RNA and DNA polymerases.\(^18\) Another clue to one aspect of zinc deficiency is the appearance of abnormal behavioral responses in zinc-deficient children, suggesting an impact on neurological development and intercellular communication.\(^14\)

In this case, much is already known about the multiple roles of zinc. Details concerning its role in the developmental process should become apparent in the near future and reveal the means whereby deficiency results in abnormality.

Manganese deficiency, also a teratogenic system, appears to affect the metabolism of such biogenic amines as central nervous system messengers, as well as superoxide dismutase and glycosyl transferase activities.

Similar analyses may be offered for silicon\(^7\) in its role as a cross-linking agent for collagen and proteoglycans. Scientists should be aware of the possibility of similar effects developing by purposeful reduction of human exposure to the "newer" essential trace elements: arsenic, nickel,
and vanadium. The point to be made is that interference with the development of control systems may have varied results—from gross tissue disorganization and deformity to biochemical transmission failures.

To deal with these types of questions, better analytic methods than are now available are clearly needed, means of better standardization, as well as the insight that would lead to their optimum use. The importance of analytic chemistry in this type of research cannot be overstated. Since overt pathological changes will generally be recognized, techniques are also needed that will indicate whether a suspect agent may be present or is likely to occur. Even when means of recognizing and measuring a suspect agent can be designed, we fail if we cannot design a means to assess the pathologic relevancy of the observation.

To identify a cause, it may be necessary to measure a product of the exogenous agent rather than the agent itself. For a deficiency, say of selenium, a measurement of the selenium-dependent enzyme, glutathione peroxidase, may suffice for the causation part of the equation. We must also seek the abnormality that is expressed by whatever techniques are appropriate, and understand how it came about.

Finally, as satisfying as the demonstration of an association between a cause and an effect may be, it remains the challenge to laboratory science to describe the mechanism of the relationship. The challenge is not merely an intellectual and experimental enterprise, but is the exciting prospect that with knowledge of how things happen, the prevention and the correction of developmental and genetic abnormalities may finally be within our grasp.

References