Meetings

Virus Etiology of Congenital Malformations

The epidemiologic discovery of the relation between maternal rubella infection and fetal abnormalities strengthened research efforts on other viral agents causing congenital malformations. On the whole this research has been unsuccessful. In an attempt to discover why and to explore the types of problems which must be overcome, a small, multidisciplinary conference was held in Bethesda, Maryland, 19–20 May 1967. The conference was chaired by Kurt Benirschke. Attendees included: Leonard Axelrod, Joseph Dancis, Heinz Eichenwald, Peter Gruenwald, Samuel Katz, George Margolis, Robert Miller, Shiela Mitchell, Paul Moorehead, Arthur Silverstein, Lon White (representing John Sever), and Gilbert Woodside.

Viral teratology is a complex problem involving as it does resistance in the mother to a particular viral agent; transmission, multiplication, or obstruction by the placenta of the virus itself or its metabolic products; susceptibility of the embryo or fetus to infection or injury with variable responses over time to such insults; and the clinical and laboratory correlation of abnormalities with proof of infection.

Viral infection of a cell has principally four effects—lytic replication, nonlytic replication without cell destruction, nonlytic replication with cell proliferation, and proliferation of cells without viral replication. Rubella, perhaps the most interesting virus from the point of view of congenital malformations, inhibits in its replicative cycle the normal protein synthesis mechanisms and the interaction of the nucleus with the cytoplasmic content of the host cell. This action results in diminished proliferation and a truncated life expectancy of the infected cell. It is perhaps because of this action that rubella can continue to damage products of conception throughout and beyond gestation. Similarly, viruses such as the Kilham rat virus or the H_1 virus of Toolan, which produce lytic replication in mitotic cells in the absence of an inflammatory response, can alter development throughout pregnancy. These viruses cause a spectrum of diseases ranging from early abortion to arrested cerebellar and renal development. Furthermore, the adult animal manifests a panleukopenia when infected with the H_1 virus. From a laboratory as well as a clinical point of view the picture is further complicated by the latency of certain viruses, vertical as well as horizontal transmission, and the possibility of increased susceptibility of pregnant women to infection or reactivation of previous infection.

Apart from establishing end-points, many of the difficulties in empirical prospective studies arise from the uncertainties surrounding the assay and interpretation of antibody levels. These may be confounded by cross reactions, by the activities of nonreplicating antigens, by presently unrecognized or unconsidered viruses, and by changing population patterns of exposure and resistance.

It is sometimes assumed that the embryo will respond to viral infection by death or malformation and that the fetus will respond with inflammation. In fact the situation is much more complex. For each mammal and each antigen there is a critical age prior to which the fetus cannot make an active antibody response. For rubella infection in man this critical age appears to be the 16th week of gestation. Once the critical age is past, and this may even be after birth, the fetus responds with antibody formation and hypersensitivity, identical to that in the adult. In man, however, there may be a deficit of complement throughout the greater part of gestation.

An antigen, such as a virus, entering the fetal circulation before immunity for this antigen has developed, may elicit no pathologic response at all. The fetus, while suffering a viremia, develops a specific immunologic suppression of its ability to respond to this particular antigen. The fetus infected after immunity develops shows typical pathology to the point of death. This may happen even with attenuated viruses crossing the placenta; fetal pathology may be not only different but more severe than adult. The mode of action of the virus as well as the time of infection will affect the type of fetal response. Nonreplicating antigens, on the other hand are catabolized in some instances and completely eliminated. Seen after competence develops they are viewed as entirely new entities.

Abnormalities of organogenesis may be difficult to distinguish from later resorption or degeneration. Indeed, the fetus may react to infection by general growth retardation rather than by localized pathology. Under conditions of fetal deprivation maturation is very much less affected than growth. In experimental animals, and the data can be duplicated for man, patterns can be established for organ weights in fetal deprivation. Thus liver and thymus are disproportionately small in deprived babies compared with premature or with normal full-term infants. Growth retardation and prematurity can both be produced by fetal disease and by specific maternal conditions. In order then to ascertain primary growth retardation it is essential that it be measured against gestational age.

The placenta must also be considered in relation to gestational age. While the fetal circulation is complete at approximately 20 days the maternal circulation requires approximately 40 and, until this occurs, it is not a complete, functioning organ. Hence, during much of fetal organogenesis it is the primordia of the placenta with which one must be concerned. Placental transfer of molecules may be by diffusion, solution, active transfer, or placental leak. In the dog, *Toxocara* worm routinely traverses the placenta which raises important questions concerning transplacental traffic.

In man the chorioallantoic placenta is the route of transfer for macromolecules; in the rodent it appears to be the yolk-sac placenta. Human rubella infection, causing inflammation and abnormality in the fetus, produces morphologic equivalents in the villus. However, pathology within the placenta, even that evoked by viral infection, does not necessarily equate with disease or abnormality in the baby. The epithelium and the vascular endothelium of the placenta act as

barriers to the transfer of molecules, but this mechanism has not been worked out satisfactorily.

The function of the normal and the abnormal placenta needs a great deal more study. Virtually nothing is known of the correlation between abnormalities in the placenta and abnormalities in the fetus. The site of origin of plasma cells and the production of antibodies in the human placenta as determined by biopsy, examination of delivered placentas, or by extrapolation from animal models, have yet to be ascertained.

One of the most promising animal models for human congenital malformations is the baboon. The gross structure of its placenta is comparable to the human. The production of phocomelia with thalidomide in doses similar to that recorded for human beings suggests that some functional aspects of embryogenesis may also be very comparable. The menstrual cycle has been well studied and precise dating of ovulation is relatively easy. Data are accumulating on normal embryogenesis in the baboon with descriptions of all stages from the blastocyst on. During the first 39 days of gestation the embryo appears identical to the human. While a healthy pregnant captive baboon is a relatively expensive, not to say large, experimental animal its disadvantages may well be offset by the possibility of extrapolating to man and by the confidence with which the pathology can be assessed.

DNA viruses may cause congenital abnormality through chromosome damage, such as breakage, rearrangement, nondisjunction or polymerization, in the gamete, in the zygote, and in the fetal somatic cells. This damage appears to begin with loss of contact inhibition of division followed, in a week or two in tissue culture, by a tremendous variation of the genome. None of these alterations are specific to viruses. Furthermore, many chromosomal aberrations appear to be of little moment. A dead skin cell sloughs, most abnormal sperm fail to fertilize, and only the chromosomal abnormalities in the ovum, perhaps, give rise to abnormalities.

Reproductive abnormalities are often best recognized epidemiologically. Using retrospective analyses testing and generating hypotheses, mitotic nondisjunction appears to be one possible common characteristic relating maternal age, increased risk of abortion, Down's syndrome and childhood leukemia. Sophisticated statistical techniques permit the evaluation of the significance of observed clusters of disease in time and in space. Simultaneous occurrence of diseases can suggest common etiologies. It can also permit observation of the onset and development of a disease such as leukemia through the identification of high-risk groups.

In considering any etiologic agent of congenital malformations one must look at the other manifestations of reproductive wastage, abortion, fetal death, and fetal infection. These may occur in the absence of direct fetal involvement, for instance, in response to change in placental function.

In the face of the hundreds of presently known viruses, potentially capable of producing teratogenic effects, precise identification of the genetic, chemical or physical mode of action of known viral teratogens would greatly assist in the identification of high-risk agents for epidemiologic and laboratory investigations. It is apparent that many different disciplines must contribute information and that new methods must be developed in seemingly unrelated fields before there will be any real breakthroughs in identifying and understanding viral etiologies of congenital malformations.

SHIELA C. MITCHELL
National Heart Institute, Bethesda, Maryland

GILBERT L. WOODSIDE
National Institute of Child Health and Human Development, Bethesda

Calendar of Events

Courses

Modern Developments in Low Speed Aerodynamics with application to VTOL, Tullahoma, Tenn., 25 Sept.–6 Oct. Fee: $300. *Deadline*: 18 Sept. (Director, Continuing Education, Univ. of Tennessee Space Institute, Tullahoma, Tenn. 37388)

Applied Transducer Workshop. Hopatcong, N.J., 30 Oct.–3 Nov. Instruction in instrumentation and process transducer performance, design, and application. Fee: $200. (Dr. S. D. Black, Center for Professional Advancement, P.O. Box 66, Hopatcong 07843)

Research and Development Management, Columbus (1st week) and Athens (2nd week), Ohio, 24 Sept.–6 Oct. For scientists and engineers with managerial responsibilties. Limited to 65 participants. (Director, Center for Management Development, College of Business Administration, Ohio University, Athens 45701)