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Fundamentals of clinical cardiology

Intrauterine virus infections and congenital heart disease

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The incidence of clinically significant congenital heart disease is approximately 5 cases per 1,000 live births.¹ Thus, about 20,000 babies with congenital heart disease will be born each year in the United States. Although great strides have been made in the development of sophisticated technology for the diagnosis and surgical correction of these defects, their etiologic basis remains largely undefined. In only about 20 per cent of major congenital defects is the cause known. Approximately 10 per cent are due to environmental causes such as virus infections, drugs, or radiation; the remaining 10 per cent are of genetic origin, resulting from familial inheritance and/or chromosomal aberrations.² The majority of congenital heart lesions are probably not the result of genetic or environmental factors acting alone, but rather the result of these factors acting in concert with one another.³ The genetically predisposed fetus is exposed to the appropriate environmental factor at a particular stage during organogenesis which leads to the development of malformations.^{3,4}

Since genetic control is unlikely to be developed to the point of practical application in the near future, the search for environmental factors would appear to be

the most profitable area for current effort. The aim would be, of course, to protect the susceptible host from the specific causative factors. The purpose of this review is to evaluate critically the present understanding of the role of intrauterine virus infections in the production of congenital heart disease. To this end we shall examine the methods utilized in establishing the association between viruses and congenital defects, the pathogenesis of the fetal infection and the mechanism of the production of defects, the currently available information concerning specific viruses which are known (rubella) or strongly suspected (Coxsackie B and mumps) etiologic agents in congenital heart disease, and the possibility that other viruses could be causative agents. The most promising areas for future research as well as the prospects for control of virus-induced congenital heart disease will be discussed. In this review "congenital defect" is used to refer to any structural abnormality present at birth which is due either to faulty development during the period of organogenesis or to damage to fetal tissues or organs which have already developed; the phrase "congenital malformation" is a more restricted term referring to an abnormality resulting from faulty organogenesis.

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Viruses as etiologic agents

There are five reasons why viruses have been considered likely causative agents in the genesis of congenital heart disease. First, there is the firmly established precedent of intrauterine infection with rubella virus during the first trimester of pregnancy leading to multiple congenital anomalies, including those of the heart. Second, viruses are ubiquitous. Approximately 5 per cent of pregnancies have been shown to be complicated by at least one definite or presumed virus infection, exclusive of the common cold.⁵ Most women might be expected to have at least one viral upper respiratory or gastrointestinal tract infection during any 9 month period. Third, the majority of virus infections in the adult are subclinical, or produce only minimal disease. Thus, a virus infection could be unrecognized in the mother, yet produce significant disease in the fetus. This situation, then, could lead to the occurrence of a "congenital defect of unknown etiology." Fourth, viruses are known to multiply readily in rapidly dividing immature cells, with resultant cell destruction or alteration of cell function.⁶ With more destructive viruses (e.g., measles or vaccinia) fetal death with abortion or stillbirth can occur. With less destructive agents (e.g., rubella or cytomegalovirus) the fetus may survive but defects are present at birth. Fifth, there are numerous examples in experimental animals where infection with a virus results in little or no disease in the pregnant mother, yet the fetuses are aborted or the newborn offspring are deformed.⁷

Maternal viral infections that do not have significant viremia as part of their pathogenesis are unlikely to pose a threat to the fetus. Although fetal death and abortion or stillbirth have resulted from indirect or toxic effects of virus infection (through disease in the mother or alteration of placental function), clinical and experimental data suggest that direct fetal infection is necessary for congenital defects to occur.^{5,6} The placenta acts as an effective barrier against most viral agents that gain access to the maternal circulation. Certain viruses, however, can infect susceptible cells of the placenta and reach the fetal circulation by growth *through* the layers of the placenta rather than by passive diffusion. Alternate

mechanisms of fetal infection could be virus-induced vascular lesions in the placenta which result in abnormal communication between maternal and fetal circulations,⁸ or diapedesis of maternal virus infected leukocytes through the placental layers to the fetal circulation.⁹⁻¹¹

In general, two different epidemiologic methods have been utilized to explore the viral etiology of congenital malformations: retrospective analysis and prospective studies.¹² The former technique was utilized by Gregg¹³ in making the association between the increased incidence of congenital cataracts and a prior rubella epidemic in Australia. The accuracy of this method depends on several factors: (1) a viral illness in pregnant women which is clinically manifest to a degree sufficient to allow a definitive etiologic diagnosis to be made in the majority of individuals infected, (2) an accurate memory on the part of the mother or a visit to a physician during the illness so that the disease is recorded, or (3) the occurrence of an epidemic due to a particular agent which is recognized by the health authorities and which, if it produces disease in the fetus, presents a uniform clinical pattern. Prospective studies are more difficult and costly to perform for several reasons: (1) because large numbers of pregnancies must be involved in order to provide a sufficient number of anomalies for statistical evaluation, (2) because the majority of virus infections during pregnancy are subclinical, acute and convalescent serum samples from the mother must be available for serologic survey of a number of viruses, (3) because the critical time for the occurrence of fetal deformities is the first trimester, ideally the mother should be enrolled in the study early in gestation so that any serum samples obtained would bracket a subsequent viral illness.

Although prospective studies are more involved than retrospective analyses, both epidemiologic approaches have nevertheless provided considerable useful information and have frequently suggested leads for further study. Important verification of the association of a given virus with a particular constellation of congenital defects has also been provided by the laboratory. The successful propagation in tissue culture of cytomegalovirus in 1956^{14,15} and of rubella

virus in 1962^{16,17} led to the use of virus isolation techniques and antibody determinations to confirm that the defects in the newborn infant were the result of intrauterine infection with those particular viruses. The epidemiologic and laboratory data available to date on each of the viral agents which are known or believed to be likely etiologic agents in congenital heart disease are summarized below.

Rubella. It has been conclusively proved that rubella virus is etiologically related to congenital heart disease. The large rubella epidemic in the United States in 1964 provided an opportunity for further confirmation of Gregg's earlier observations.¹³ This association was documented not only by the occurrence of clinical rubella in the woman with early gestation, but more importantly by virus isolation and/or the demonstration of a significant rise (fourfold or greater) in rubella antibody titer during the course of the pregnancy. Virus was also isolated from fetal tissues obtained following abortion or stillbirth, from the newborn infant immediately after birth, and from the throat and other sites of involved infants for many months after birth. Specific anti-rubella antibodies are produced by the congenitally infected fetus and infant. IgM antibodies, which are not passed transplacentally from the mother to the fetus, are present in umbilical cord blood or in blood obtained from the newborn infant shortly after birth. IgG antibodies produced by the infant are demonstrable in the serum over 6 months of age, a time after which maternal transplacentally transmitted IgG would no longer be present.¹⁸

The clinical manifestations of the congenital rubella syndrome are well described and include low birth weight due to intrauterine growth retardation, cardiac malformations, cataracts and microphthalmia, mental retardation, hepatosplenomegaly, thrombocytopenic purpura, and lesions in the long bones.⁵ The incidence of congenitally acquired rubella is approximately 1 case per 1,000 live births during nonepidemic periods (pre-rubella vaccine data), but rose to 7 cases per 1,000 births during the 1964 epidemic. The frequency of all malformations is highest when the maternal infection occurs early in pregnancy—a 10 to 50 per cent malformation rate with infec-

tion in the first month, a 14 to 25 per cent malformation rate in the second month, and a 7 to 17 per cent malformation rate in the third month.⁵ Congenital heart disease occurred in 48 per cent of a series of 376 children with rubella syndrome.¹⁹ The most common cardiac lesions in 87 catheterized patients were: patent ductus arteriosus in 78 per cent, right pulmonary artery stenosis in 70 per cent, left pulmonary artery stenosis in 56 per cent, valvular pulmonic stenosis in 40 per cent, mild aortic valvular stenosis in 14 per cent, aberrant subclavian artery in 11 per cent, and ventricular septal defect in 10 per cent. It is clear that the majority of cardiac malformations occur when rubella infects the mother during the first trimester, although pulmonary artery stenosis has been reported following rubella infection after the first trimester.²⁰ It has been estimated that maternal rubella may account for between 1 and 2 per cent of all malformations of the heart.¹

Rubella is the only viral agent which has been proved to be a true teratogen; that is, which results in congenital *malformations*. Hence, knowledge of the mechanisms by which rubella causes deformities may lead to a more basic understanding of the pathogenesis of congenital malformation of the heart. Rubella is a noncytolytic virus in certain tissues; that is, it does not destroy the cells in which it replicates. This characteristic would tend to allow survival of the infected fetus, yet result in disordered function of cells, tissues, and organs. On the other hand, selective cell destruction may occur. In pathologic studies of therapeutically aborted rubella infected fetuses, scattered foci of necrotic cellular damage without inflammatory infiltrate were noted in endothelial cells of blood vessels and in myocardial cells.⁸ These rubella-induced defects could result in the defective form and/or function of the developing tissue by direct cellular destruction or hypoxic damage secondary to blood vessel obliteration. Alteration of the elastic or muscle fibers in the ductus arteriosus, for example, could be the reason for the failure of postnatal ductus closure. Studies with tissue obtained from infants with rubella syndrome and maintained in culture revealed that the cells were persistently infected with rubella

virus and had a decreased growth rate and shortened survival time.²¹ Naeye and Blanc²² noted that the growth retardation in infants with the rubella syndrome was the result of a decreased number of cells in the organs. This impaired cellular growth, if it occurred during a crucial phase in cardiac development, could well result in such cardiac anomalies as septal defects. Increased numbers of chromosome breaks have been noted in leukocyte cultures of children with congenital rubella.²³ It is possible that this chromosomal injury results in cell loss (due to impaired DNA replication) during rapid organ development and is in part responsible for the congenital anomalies.

Transplacental infection with rubella virus has been demonstrated in the monkey, ferret, rabbit, and rat.⁷ However, the complete rubella syndrome as it occurs in humans, particularly with regard to cardiac defects, has not been duplicated in experimental animals.^{7,24} The neonatal rat is the only animal that has shown evidence of cardiac damage following gestational rubella.²⁵ Pathologic sections of the involved hearts revealed disrupted cords of cardiac muscle and septa, with scattered eosinophilic areas replacing cardiac tissue. Rubella virus antigen was demonstrated by immunofluorescence techniques in these same areas. There was no mention of gross cardiovascular defects.

There are many unanswered questions concerning the pathogenesis of congenital rubella. Why does the virus damage cells of the myocardium, lens, and inner ear in the fetus but not in the adult? Are similar pathogenic mechanisms instrumental in the production of cardiovascular anomalies and in producing damage to the lens? Is the disturbance in organogenesis primarily a matter of cell death or are more subtle derangements in cell function operative? The answers to these questions depend upon an increased understanding of the molecular biology of virus-cell interaction obtained through *in vitro* studies of human tissue and the use of animal models.

Coxsackie B virus. There is strong circumstantial evidence that the Coxsackie B group of viruses may be etiologically associated with congenital heart disease. These viruses are the most common agents known

to cause myocarditis and pericarditis in children and adults, thus demonstrating their strong cardiotropic potential.²⁶ Transplacental infection of the late gestation fetus has been reported with disseminated disease presenting in the newborn infant as myocarditis, hepatitis, and meningoencephalitis.⁵ Brown and Evans,²⁷ in a prospective study, demonstrated the significant association between serologic evidence of Coxsackie B3 and B4 virus infection in mothers during the first trimester of pregnancy and the birth of infants with various types of congenital heart disease. Approximately half of the maternal infections were completely subclinical; in those cases where symptoms occurred, none would have permitted a specific clinical diagnosis. There was no difference between mothers of infants with congenital abnormalities and matched control mothers of normal infants with regard to the incidence of infection with other Coxsackie virus types B1, B2, B5, and A9, with ECHO virus types 6 and 9, with influenza virus types A and B, or with the adenovirus group. There was no serologic evidence of rubella in any of the mothers who gave birth to infants with heart anomalies. Virus isolation and antibody studies were not done on the infants. Burch and co-workers²⁸ demonstrated the presence of Coxsackie B virus antigen by immunofluorescence techniques in myocardial tissue obtained at routine autopsies from infants and children. Twenty-nine of 50 hearts examined showed interstitial myocarditis; in 12 of these, specific antigen was demonstrated. Five infants in this latter group either were stillborn or died within hours after birth, further documenting the transplacental passage of this group of agents. None of these five infants, however, had evidence of congenital heart disease. In a previous publication by this same group of investigators,²⁹ Coxsackie B antigen was demonstrated in the myocardium of an infant who died at 2 days of age and who had a widely patent ductus arteriosus. Bates³⁰ described a stillborn infant with calcific pancarditis and hydrops fetalis in whose myocardium specific Coxsackie B3 virus antigen was demonstrated. No gross cardiovascular anomaly was noted. In contrast to rubella, no large-scale epidemic has been reported with Coxsackie

B viruses which was associated with an increased incidence of congenital heart disease.¹² An animal model for Coxsackie B virus infection and congenital heart disease has not been developed. The virus is transmitted transplacentally in the mouse; fetal infection and death results, but no anomaly.³¹ The evidence then for Coxsackie B virus etiology of congenital heart lesions is strongly suggestive but not yet positive.

Additional information or studies which would provide further evidence for the association include: (1) confirmation by other laboratories of the provocative serologic studies of Brown and Evans,²⁷ (2) the demonstration of specific IgM antibodies in umbilical cord or neonatal sera directed against the Coxsackie B viruses in significant numbers of infants with congenital heart lesions as compared with control infants, (3) the isolation of Coxsackie B viruses from significant numbers of newborn infants with congenital heart disease as compared with the isolation from normal control infants, and (4) the identification of Coxsackie B virus antigen in the myocardium and/or cardiac lesions of a significant number of infants who died with congenital heart disease as compared with infants who died from noncardiac and noninfectious causes.

Mumps. The issue of the relationship between intrauterine mumps virus infection and postnatal endocardial fibroelastosis (EFE) remains controversial.³²⁻³⁵ The clinical evidence supporting this relationship is the occurrence of a significantly higher incidence of skin test positivity to mumps antigen in patients with EFE than in matched controls. However, mumps virus has not been isolated from these patients, and mumps antibody has usually not been present in their serum. For the newborn infant to have a positive skin test without the presence of virus or antibody, the fetus must: (1) recover from the virus infection *in utero* so that the virus cannot be isolated after birth, and (2) manifest a cellular, but not humoral, immune response against the mumps virus antigen so that delayed hypersensitivity, but not antibody, is demonstrable. It is appropriate to examine the available experimental data concerning the fetal response in order to determine the validity of these hypotheses.

Fetal recovery from intrauterine virus infection has been documented and transplacental passage of maternal antibody may play a role in this recovery process.⁶ Development of a cellular immune response without a humoral immune response in the fetus could occur by one of several mechanisms: (1) a tolerance could develop with respect to humoral but not cellular immunity, (2) the virus infection could occur at such a time during the ontogeny of the immune response in the fetus that the capacity to express cellular immunity was present but not the ability to produce antibody, and (3) maternal passive antibody could inhibit humoral, but not cellular, immunity. Most experimental evidence would argue against the first two mechanisms. In the animal models where antibody tolerance to intrauterine virus infection exists, there has been demonstrable persistence of virus in the circulating blood and tissues.^{6,35} As noted, mumps virus has not been isolated from patients with EFE. In most mammalian species where the ontogeny of the immune response has been examined, the capacity to produce antibody develops prior to the ability to reject skin grafts or to manifest skin-test-delayed hypersensitivity.³⁶ The postulated mumps-EFE association requires that delayed hypersensitivity develop prior to antibody production. The inhibition of antibody production by passive antibody administration has been shown to occur³⁶; this mechanism therefore remains a possibility.

There are additional difficulties in the attempts to associate intrauterine mumps with EFE. The accuracy of the mumps skin test (on which the relationship with EFE rests) in predicting individuals with prior exposure to mumps virus has been questioned.³⁷ Of particular importance is the fact that individuals with positive skin tests have contracted mumps, indicating that false positive tests can occur. In addition, children with EFE have contracted mumps.³⁵ In the consideration of maternal gestational mumps, the available clinical information does not support the mumps-EFE relationship. Although some prospective studies have shown an increased incidence of abortion and stillbirth following gestational mumps, most have not demonstrated an increase in congenital malforma-

tions.^{2,12,38} In the few case reports reviewed by Hyatt³⁹ of congenital abnormalities in infants whose mothers had mumps during pregnancy, a variety of types of defects occurred. Cardiac lesions were infrequent and EFE was not specifically mentioned. There has been little or no evidence that mumps infection occurred during pregnancy in the mothers of infants who developed EFE.³⁵

However, in support of the mumps virus etiology of EFE, there has been one case report⁴⁰ of acquired myocarditis and autopsy-proved endocardial fibroelastosis following mumps virus infection in a 19-month-old child. In addition, there are experimental animal data to add to the evidence. Infection of chick embryos with mumps virus during the very early phases of differentiation resulted in persistent virus infection, particularly of the heart and brain, and pathologic evidence of interstitial myocarditis in the late gestation embryo.⁴¹ Chicks at one year of age showed evidence of EFE. Antibody was demonstrated by one month of age in the chicks, but delayed hypersensitivity to mumps virus could not be elicited. Direct inoculation of first trimester fetal monkeys with mumps virus resulted in virus replication which was controlled and led to the development of cellular immunity but no detectable humoral immunity in the newborn animals.⁴² To date, cardiac pathology has not been seen in the infected monkeys. Thus, the chick embryo model provides evidence that gestational mumps can cause EFE and the monkey fetus model suggests that intrauterine mumps can result in cellular, but not humoral immunity in the newborn.

It would appear that there is suggestive evidence that gestational mumps is etiologically related to EFE; however, as noted, there is conflicting evidence. Further studies will be required before the relationship is conclusively proved or disproved. Additional information which would be helpful in establishing the association includes: (1) prospectively obtained virologic (virus isolation) or serologic (antibody titer rise) data that maternal mumps is associated with the birth of offspring who develop EFE, (2) the demonstration of mumps virus antigen in the myocardium or endo-

cardium of patients with EFE and not in the heart tissue of patients the same age who died from noncardiac causes, and (3) the demonstration that EFE can result in monkeys and other mammalian fetuses from the infection with mumps virus.

Other viruses. The only viral agents which have been conclusively proved to cause congenital defects are rubella and cytomegalovirus. Cardiovascular anomalies, however, are infrequent findings in infants with congenital cytomegalovirus infection.^{5,43} It is not known whether the cardiac defects are a coincidental occurrence or the direct result of the intrauterine virus infection. Influenza viruses, and recently herpes simplex virus, have been incriminated by some studies as causing congenital defects, but the abnormalities reported have usually been of the central nervous system.^{2,3,5,12} ECHO viruses might be considered likely candidates because infections are prevalent and frequently subclinical. However, several documented ECHO virus epidemics have not resulted in an increased incidence of abnormal offspring.^{2,12} Rhinoviruses, the most frequent causative agents of the common cold, usually result in infections limited to the respiratory tract and would not be expected to cause fetal infection. The recently isolated (by means of tracheal organ culture techniques) and described human coronaviruses also appear to cause mild or asymptomatic infections limited to the respiratory tract.⁴⁴ These new organ culture techniques (e.g., human fetal gut organ culture⁴⁵), may result in the isolation and characterization of new viral agents associated with human disease which could have a role in the production of congenital malformations. Infections with reoviruses are relatively common and usually asymptomatic, but may have an associated viremia.⁴⁶ Although reovirus type 1 has been shown to cause transplacental infection and abnormal offspring in mice,⁴⁷ to the writer's knowledge the role of this group of agents in human intrauterine virus infection has not been examined. The viruses which are known to cause transplacental infection and congenital defects in experimental animals⁷ have been considered as possible agents in humans, but their precise role has not been defined.⁴⁸ It is apparent that the list of viral agents which could

cause congenital heart disease is long. It is not possible to predict which viruses are likely to cause fetal abnormalities from the diseases that these viruses produce in children and adults. Fifty years ago who would have predicted that rubella or cytomegalovirus infections would be significant factors in the genesis of congenital defects? It becomes obvious, then, that the task of identifying specific viruses as etiologic agents in the entire spectrum of congenital heart disease will not be an easy one.

Future efforts

The areas of research which would appear to hold the greatest promise for future investigation fall into one of four categories: (1) the epidemiology of congenital heart disease, (2) the association of maternal viral infection with abnormal offspring, (3) the in-depth virologic investigation of the infant with a cardiac defect, and (4) the development of experimental animal models of congenital heart disease.

More precise epidemiologic surveillance data are needed to determine two aspects of congenital heart disease: (1) the frequency of occurrence of the various types of defects in different populations, and (2) the effect of race, geography, climate, or season of the year on the occurrence of these defects. The correlation between recognized patterns of epidemiology and the pathogenesis of infectious diseases and the occurrence of congenital heart disease will provide leads for the possible association of particular viral agents with specific heart lesions and will suggest areas for further in-depth study. For example, an association has been reported between seasons of the year and the birth of children with patent ductus arteriosus.¹ A search for possible causative viruses, by both epidemiologic and laboratory means, could focus initially on those agents known to be seasonally prevalent at the time of or shortly after conception in these pregnancies. Other epidemiologic factors such as (1) the frequency of infection in women of childbearing age with the suspected viruses, and (2) the occurrence of viremia as part of the pathogenesis of the disease in the mother can be utilized to narrow the spectrum of viral agents even further.

In the consideration of future efforts to

associate specific viral agents with particular constellations of congenital defects, it is appropriate to examine the approaches utilized in the establishment of rubella and cytomegalovirus as causative agents of congenital abnormalities. Although the documentation of maternal infection during pregnancy and the association with abnormal offspring will provide important suggestive information, proof of the association must come from evidence of infection in the abnormal newborn. With all the negative data from previous reports, it is unlikely that clinically recognized viral infections during pregnancy are a major cause of congenital malformations. Thus, for the reasons noted above, the most productive information concerning maternal infection will likely come from large prospective studies.

Extensive virus isolation and serologic studies should be attempted in defective neonates where the etiology is not readily apparent. In congenital rubella and cytomegalovirus infections and in virtually all of the animal models of intrauterine virus infections, either virus or antibody or both are demonstrable in the newborn. By focusing on the abnormal newborn, the investigator can be more selective in terms of numbers of patients and he can proceed with more in-depth studies. One can question, however, whether the absence of a demonstrable viral agent or of a specific antibody in the newborn infant can, in fact, rule out an intrauterine virus infection. The obvious exception would be when the infection is due to a new viral agent which had not previously been isolated or to a known agent which would not grow in the cell culture systems routinely utilized for virus isolation. The approach to this problem would, of course, be the application of the new, not previously utilized, techniques available for culturing and identifying viral agents. The success of this approach requires that there be a persistent infection in the fetus so that the virus is present in the excretions or tissues of the newborn infant and/or that a fetal humoral immune response occur which results in antibody being present in the neonate.

It is theoretically possible, however, that intrauterine virus infection could occur, but that neither virus nor antibody would

be demonstrable in the newborn infant even if techniques appropriate for that virus were utilized. The experimental data which would support or refute this hypothesis should be examined. A virus could infect the early gestation fetus and result in tissue destruction and/or alteration of organogenesis, yet be eliminated prior to birth. Recovery of the fetus from the infection could occur through the production and action of interferon, of maternal transplacental antibody, or through other non-specific factors developed by the mother or through processes occurring in her, in the placenta, or in the fetus.^{6,49} There are good clinical and experimental data to indicate that the fetus may be capable of controlling certain virus infections *in utero*, but the mechanisms responsible are poorly understood.^{6,20,42,49} Absence of antibody in the newborn infant, even though there had been exposure to an antigen (virus) *in utero*, was shown to be possible in the discussion of the mumps-EFE hypothesis. Studies in the fetal lamb have shown that challenge of the immunologically immature fetus with an antigen will not elicit a detectable antibody response.^{36,48} Challenge of that same fetus with the same antigen at a later gestational age or after birth at a time when it has developed the capacity to respond immunologically, results in a primary antibody response identical to that which would be expected if the animal had never been exposed to the antigen. In other words, there was no detectable primary antibody response to the antigen in the early gestation fetus, there was no evidence of a secondary or anamnestic response when the fetus was rechallenged with the same antigen, and there was no evidence of immunologic tolerance to the antigen. Each of these three parameters has been utilized to determine prior exposure to an antigen, yet, as noted, none may be present in the newborn animal which received an antigenic challenge early in gestation.

Further work needs to be done to determine if virus infection of the early gestation fetus can result in a similar situation—recovery from the infection but no humoral evidence of contact with the virus antigen. The studies of St. Geme and associates^{41,42} with mumps virus infection of the fetal monkey are important in this regard. New-

born monkeys were shown to have recovered from the virus infection *in utero* in the absence of detectable mumps antibody. Administration of mumps virus postnatally to the monkeys infected *in utero* elicited a normal primary antibody response.⁵⁰ These studies need to be extended to other viral agents suspected of causing congenital defects in humans. The use of animal models other than primates has been and should still be considered, since this species is expensive and technically difficult to work with.

If neither virus isolation nor antibody demonstration is possible in the newborn infant who had an intrauterine infection, then other means will have to be utilized. Frequently, viral antigen can be demonstrated in tissues by fluorescent antibody techniques when infectious virus cannot be recovered from the same tissues. The important observations of Burch and co-workers^{28,29} concerning Coxsackie B virus antigen in myocardial tissues should be extended to other viruses. Tissue obtained at the time of cardiac surgery, as well as from autopsy material, could be utilized for these studies. It will be important, however, to demonstrate that this is a reliable technique for the association of specific viral agents with congenital heart lesions. For example, rubella virus antigen should be demonstrable in excised ductus tissue in infants with culture-proved congenital rubella syndrome who are undergoing ligation of the ductus.

The use of experimental animals will provide the opportunity to determine if viral agents suspected of causing abnormalities in humans can produce defects in animals. In addition, if congenital defects produced in animal models are similar to those found in humans, this would permit the elucidation of mechanisms responsible for the genesis of these defects. Studies should be performed not only with human viruses, but also with viral agents which are natural to that particular animal species.

To the writer's knowledge, a good mammalian animal model for congenital heart disease is not available. The microscopic lesions noted in the cardiac muscle of fetal rats infected with rubella virus were mentioned above.²⁵ Ectopic hearts were occasionally noted in hamster embryos infected

following intravenous inoculation of the pregnant female with H-1 virus.⁵¹ The ideal natural virus model infection would be one in which the virus infected the mother, causing little or no disease, was passed transplacentally, and resulted in infection of the fetus with the production of defects.

Much of the work on fetal infection to date has been of a descriptive nature—i.e., which viruses can infect and cross the placenta, which viruses infect and damage the fetus, the time during gestation that maternal infection occurs which then results in fetal infection and disease, and the pathologic nature of the fetal disease. Much remains to be learned about the mechanisms of resistance to virus infection in the fetus and how these mechanisms differ from similar mechanisms in the adult. We need to learn the basis for the enhanced susceptibility of the fetus to virus infection and what the role of the placenta is, not only in preventing or in permitting transmission of virus to the fetus, but also in assisting recovery from infection once the virus has reached the fetus.

Prospects for control

The likelihood of developing effective means of preventing virus-induced congenital heart disease in humans will depend on the results of the studies on etiology and on the pathogenesis of infections in the fetus. If only a few viruses are identified as causative agents, then the production of vaccines for immunization would be a feasible goal. The development of rubella vaccine is a good example of successful efforts in this area. However, the mere identification of a teratogenic agent does not mean that successful vaccine production will ensue. For example, the prospects for a cytomegalovirus vaccine do not appear bright. If the number of causative agents is large, vaccine production would not be a practical solution and antiviral chemotherapy would provide an alternate approach. Rapid and efficient means would have to be developed to provide an accurate etiologic diagnosis of virus infections during the early gestational period in the woman. Once infection with a teratogenic agent was identified, safe and effective antiviral drugs would be needed for treatment of the infection in the mother and in the fetus. A great

deal of work has been done and is currently in progress on the use of interferon⁵² and interferon inducers⁵³ in the treatment of human viral disease. Certainly further work is necessary to check the efficacy of these substances in experimental animals with a number of different types of virus infections before their use in human viral disease can be expected. However, limited clinical trials have been cautiously undertaken. As will be obvious, both the vaccine and the antiviral chemotherapeutic approach to the prevention and/or control of congenital heart disease is still a dream of the future. It is only through the persistent and combined efforts of both clinical and laboratory research that sufficient information will become available to allow attempts to be made toward practical solutions of the problem.

Summary

The etiologic basis for the vast majority of cases of congenital heart disease remains largely undefined. Viruses have been considered to be likely candidates since the recognition of the association between intrauterine rubella and congenital heart disease. Although the pathogenesis of cardiovascular defects is poorly understood, information gained from the study of congenital rubella syndrome suggests that mechanisms such as focal endothelial cell damage, resulting in obliteration of vascular supply, decreased growth rate, and shortened survival time of certain cells, and disturbed DNA replication in cells whose chromosomes were damaged secondary to the effects of virus replication may be operative in the production of defects in the developing fetus. In addition to rubella there is suggestive, but not conclusive, evidence that Coxsackie B3 and B4 virus infections during pregnancy can result in the birth of infants with a variety of types of congenital heart lesions and that intrauterine mumps virus infection may be etiologically related to the postnatal development of endocardial fibroelastosis (EFE). Although there are a number of other viruses that are potential etiologic agents of congenital heart disease, the current status of information is inadequate to allow even suggestive associations to be made. The most profitable areas for future inves-

tigation appear to be: (1) the epidemiology of congenital heart disease, (2) prospective studies of the association of maternal viral infection with abnormal offspring, (3) the in-depth virologic investigation of the infant with a cardiac defect, and (4) the development of experimental animal models of congenital heart disease. Successful control of virus-induced congenital heart disease will depend on the results of these investigations and the development of vaccines against the identified causative viruses and/or safe and effective antiviral chemotherapy for the woman in early gestation who is infected with a known teratogenic agent.

REFERENCES

- Higgins, I. T. T.: The epidemiology of congenital heart disease, *J. Chronic Dis.* **18**:699, 1965.
- Dudgeon, J. A.: Congenital defects: Virus infections, *Proc. R. Soc. Med.* **61**:995, 1968.
- Jackson, B. T.: The pathogenesis of congenital cardiovascular anomalies, *New Engl. J. Med.* **279**:25, 80, 1968.
- Campbell, M.: Causes of malformations of the heart, *Br. Med. J.* **2**:895, 1965.
- Overall, J. C., Jr., and Glasgow, L. A.: Virus infections of the fetus and newborn infant, *J. Pediatr.* **77**:315, 1970.
- Mims, C. A.: Pathogenesis of viral infections of the fetus, *Progr. Med. Virol.* **10**:194, 1968.
- Elizan, T. S., and Fabiyi, A.: Congenital and neonatal anomalies linked with viral infections in experimental animals, *Am. J. Obstet. Gynecol.* **106**:147, 1970.
- Tondury, G., and Smith, D. W.: Fetal rubella pathology, *J. Pediatr.* **68**:867, 1966.
- Desai, R. G., and Creger, W. P.: Maternofetal passage of leukocytes and platelets in man, *Blood* **21**:665, 1963.
- Gresser, I., and Lang, D. J.: Relationships between viruses and leukocytes, *Progr. Med. Virol.* **3**:62, 1966.
- Jack, I.: Leukocyte viremia and intrauterine infection, *AM. HEART J.* **80**:291, 1970.
- Brown, G. C.: Recent advances in the viral aetiology of congenital anomalies, *Adv. Teratol.* **1**:55, 1966.
- Gregg, N. M.: Congenital cataract following German measles in the mother, *Trans. Ophthalmol. Soc. Aust.* **3**:35, 1941.
- Rowe, W. P., Hartley, J. W., Waterman, S., Turner, H. C., and Huebner, R. J.: Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids, *Proc. Soc. Exp. Biol. Med.* **92**:418, 1956.
- Smith, M. G.: Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus disease, *Proc. Soc. Exp. Biol. Med.* **92**:424, 1956.
- Weller, T. H., and Neva, F. A.: Propagation in tissue culture of cytopathic agents from patients with rubella-like illness, *Proc. Soc. Exp. Biol. Med.* **111**:215, 1962.
- Parkman, P. D., Buescher, E. L., and Arntstein, M. S.: Recovery of rubella virus from army recruits, *Proc. Soc. Exp. Biol. Med.* **111**:225, 1962.
- Krugman, S.: International conference on rubella immunization. I. Rubella as a disease. II. Virology and epidemiology of rubella, *Am. J. Dis. Child.* **118**:1, 1969.
- Cooper, L. Z., Ziring, P. R., Ockerse, A. B., Fedun, B. A., Kiely, B., and Krugman, S.: Rubella: Clinical manifestations and management, *Am. J. Dis. Child.* **118**:18, 1969.
- Hardy, J. B., McCracken, G. H., Gilkeson, M. R., and Sever, J. L.: Adverse fetal outcome following maternal rubella after the first trimester of pregnancy, *J.A.M.A.* **207**:2414, 1969.
- Rawls, W. E., and Melnick, J. L.: Rubella virus carrier cultures derived from congenitally infected infants, *J. Exp. Med.* **123**:795, 1966.
- Naeye, R. L., and Blanc, W.: Pathogenesis of congenital rubella, *J.A.M.A.* **194**:1277, 1965.
- Nusbacher, J., Hirschhorn, K., and Cooper, L. Z.: Chromosomal abnormalities in congenital rubella, *New Engl. J. Med.* **276**:1409, 1967.
- Kono, R., Hayakawa, Y., Hibi, M., and Ishii, K.: Experimental vertical transmission of rubella virus in rabbits, *Lancet* **1**:343, 1969.
- Bohigian, G. M., Fox, J., and Cotlier, E.: Immunofluorescent localization of rubella virus in the lens, retina and heart of congenital rubella-infected rats, *Am. J. Ophthalmol.* **65**:196, 1968.
- Lerner, A. M.: Coxsackievirus myocardopathy, *J. Infect. Dis.* **120**:496, 1969.
- Brown, G. C., and Evans, T. N.: Serologic evidence of coxsackievirus etiology of congenital heart disease, *J.A.M.A.* **199**:183, 1967.
- Burch, G. E., Sun, S. C., Chu, K. C., Sohal, R. S., and Colcolough, H. L.: Interstitial and Coxsackievirus B myocarditis in infants and children, *J.A.M.A.* **203**:1, 1968.
- Burch, G. E., Sun, S. C., Colcolough, H. L., Sohal, R. S., and DePasquale, N. P.: Coxsackie B viral myocarditis and valvulitis identified in routine autopsy specimens by immunofluorescent techniques, *AM. HEART J.* **74**:13, 1967.
- Bates, H. R.: Coxsackie virus B3 calcific pancarditis and hydrops fetalis, *Am. J. Obstet. Gynecol.* **106**:629, 1970.
- Surjus, A.: Effects of Coxsackie B3 virus on pregnant mice and its transplacental transmission, *Ann. Inst. Pasteur* **100**:825, 1961.
- Noren, G. R., Adams, P., and Anderson, R. C.: Positive skin reactivity to mumps virus antigen in endocardial fibroelastosis, *J. Pediatr.* **62**:604, 1963.
- St. Geme, J. W., Jr., Noren, G. R., and Adams, P., Jr.: Proposed embryopathic relation between mumps virus and primary endocardial fibroelastosis, *New Engl. J. Med.* **275**:339, 1966.
- Gersony, W. M., Katz, S. L., and Nadas, A. S.: Endocardial fibroelastosis and the mumps virus, *Pediatrics* **37**:430, 1966.
- Katz, S. L.: The possible relationship of viruses,

- other than rubella and cytomegalovirus, to the etiology of birth defects, *in* Bergsma, D., editor: Intrauterine infections, New York, 1968, Birth Defects Original Article Series, National Foundation.
36. Sterzl, J., and Silverstein, A. M.: Developmental aspects of immunity, *Adv. Immunol.* **6**:337, 1967.
 37. Brunell, P. A., Brickman, A., O'Hare, D., and Steinberg, S.: Ineffectiveness of isolation of patients as a method of preventing the spread of mumps, *New Engl. J. Med.* **279**:1357, 1968.
 38. Siegel, M., and Fuerst, H. T.: Low birth weight and maternal virus diseases, *J.A.M.A.* **197**:680, 1966.
 39. Hyatt, H. W.: Relationship of maternal mumps to congenital defects and fetal deaths, and to maternal morbidity and mortality, *Am. Practit.* **12**:359, 1961.
 40. Carstens, P. H. B.: Postnatal mumps virus infection associated with endocardial fibroelastosis, *Arch. Pathol.* **88**:399, 1969.
 41. St. Geme, J. W., Jr., Peralta, H., Farias, E., Davis, C. W. C., and Noren, G. R.: Experimental gestational mumps virus infection and endocardial fibroelastosis, *Pediatrics* **48**:821, 1971.
 42. St. Geme, J. W., Jr., Davis, C. W. C., and Van Pelt, L. F.: A primitive immunologic marker of intrauterine virus infection, Presented to The Society for Pediatric Research, Atlantic City, 1971.
 43. McCracken, G. H., Shinefield, H. R., Cobb, K., Rausen, A. R., Dische, R., and Eichewald, H. F.: Congenital cytomegalic inclusion disease, *Am. J. Dis. Child.* **117**:521, 1969.
 44. McIntosh, K., Kapikian, A. Z., Turner, H. C., Hartley, J. W., Parrott, R. H., and Chanock, R. M.: Seroepidemiologic studies of coronavirus infection in adults and children, *Am. J. Epidemiol.* **91**:585, 1970.
 45. Dolin, R., Blacklow, N. R., Malmgren, R. A., and Chanock, R. M.: Establishment of human fetal intestinal organ cultures for growth of viruses, *J. Infect. Dis.* **122**:227, 1970.
 46. Tillotson, J. R., and Lerner, A. M.: Reovirus type 3 associated with fatal pneumonia, *New Engl. J. Med.* **276**:1060, 1967.
 47. Hassan, S. A., and Cochran, K. W.: Effects of reovirus type 1 on the developing mouse, *Am. J. Pathol.* **55**:147, 1969.
 48. Woodside, G. L., and Mitchell, S. C.: Viral etiology of congenital malformations, U. S. Dept. of Health, Education, and Welfare, 1968.
 49. Baron, S.: Mechanism of recovery from viral infection, *Adv. Virus Res.* **10**:39, 1963.
 50. St. Geme, J. W., Jr.: Personal communication.
 51. Ferm, V. H., and Kilham, L.: Congenital anomalies induced in hamster embryos with H-1 virus, *Science* **145**:510, 1964.
 52. Finter, N. B.: Exogenous interferon in animals and its clinical implications, *Arch. Intern. Med.* **126**:147, 1970.
 53. Hilleman, M. R.: Double-stranded RNAs (Poly I:C:) in the prevention of viral infections. *Arch. Intern. Med.* **126**:109, 1970.