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Teratogenic Effect of Rubella Virus

Thirty years have now elapsed since the 1940 epidemic of rubella in Australia and the recognition the following year by the late Sir Norman Gregg of a number of cases of congenital cataracts and other congenital defects. Much has been written about rubella and its effect on the fœtus since, but the more one reads Gregg's own accounts the more one is impressed by the careful and precise way in which he recorded his observations (Gregg 1941, 1944). Many of the manifestations reported in 1964 as 'new' are to be found in his papers. Despite the amount that has been written and spoken about rubella, especially congenital rubella, in recent years, there are a number of reasons why a further communication seems justified at the present time.

In the first place rubella virus is the only infectious agent of man which is strictly teratogenic, that is to say capable of causing congenital malformations. The effect of rubella virus is more extensive than this, and forms of fœtal damage occur which cannot be strictly classified as malformations. Nevertheless, it was initially the damage to the developing eye, heart and ear which drew attention to the pathogenic properties of this otherwise innocuous agent, and, understandably, any transmissible agent that had such an effect warranted close attention.

For three decades or more after Gregg's observations in 1941 progress in this field was restricted to epidemiological observations on the incidence of malformations after maternal rubella in pregnancy. Experimental studies were limited to a study of the disease in human volunteers, so that when, in 1962, reports were made of the isolation of rubella virus by cell culture techniques hopes were raised that laboratory techniques for the study of rubella would soon be developed. It was fortunate that new techniques for the estimation of rubella virus and antibody had been developed, before rubella appeared in epidemic form in Europe and even more extensively in the United States.

Between 1963 and 1965 many thousands of congenitally deformed children were born into the world. In the USA alone it has been estimated that 20,000 or more congenital rubella infants, many with multiple handicaps, were born. The cost of this tragedy ran into millions of dollars.

Table 1
Laboratory diagnosis of congenital rubella

Virus and viral antigen	*Rubella antibody*	*Other immunological tests*
(*a*) Isolation of virus from throat, urine and fœtal organs	Persistence of antibody	Immunoglobulin estimations of IgM in cord blood and early weeks of life
(*b*) Isolation from trypsinized tissues		
(*c*) Organ cultures	Specific IgM antibody	Lymphocyte transformation to PHA and other antigens
(*d*) Detection of viral antigen by fluorescence and electron microscopy		

The only consolation from this human tragedy is that laboratory facilities had been developed before the epidemic started and have since been further developed and improved upon. Out of this tragic human situation has arisen a better understanding of what rubella virus does to the fœtus by applying new virological and immunological techniques to the study of the pathogenesis of fœtal infection. The information stems from many sources and from comparatively few cases, a few hundred or more. Inevitably some questions remain unanswered. Some of these I shall try to define here.

Prevention is the second reason why a discussion on congenital rubella is pertinent at the present time. Vaccines have been developed and one has already been licensed for use in this country. The ultimate test of the efficacy of rubella vaccine is that congenital rubella defects should decline and then, depending on the scope of the immunization programme, either disappear or be reduced to a very low level. In assessing this, account will have to be taken of pregnancies terminated on the grounds of exposure to rubella, but one way or another it should be possible to prevent these defects.

Three aspects of congenital rubella which have a direct or indirect bearing on the nature of fœtal damage are discussed in the present paper. First laboratory methods which are currently used for the study of rubella infection of the fœtus and of infants with congenital rubella are briefly described. Secondly the clinical manifestations are listed, in order to correlate these with the underlying pathology. Thirdly the pathogenesis of both postnatal and prenatal rubella is discussed, to try to show what is known about the way in which the virus reaches the fœtus and the way in which damage is caused once the virus reaches the fœtal tissues.

LABORATORY METHODS

The laboratory methods used in the study of rubella-infected fœtuses and congenital rubella patients can be considered under three headings, and are summarized in Table 1: (1) Demonstration of infectious virus and viral antigen. (2) Estimation of rubella antibody. (3) Immunoglobulin and other immunological studies.

(1) *Demonstration of Virus and Antigen*

(*a*) *Virus isolation:* Initially, it appeared that rubella virus could only be cultured with great difficulty, but more recent work has shown that many different types of cell culture are susceptible to the virus and in many cases a cytopathic change is produced which is readily identifiable by microscopy (McCarthy & Taylor-Robinson 1967).

(*b*) *Trypsinized cultures:* Virus can be isolated by direct inoculation of the cultures mentioned above. Recent work by Rawls *et al.* (1968) has shown that better results are obtained by inoculation of a trypsinized suspension of fœtal organs or tissues.

(*c*) *Organ cultures:* This technique has been used by Banatvala *et al.* (1969) for studying the location of virus multiplication in infected organs.

(*d*) Rubella virus antigen can also be demonstrated in infected tissues by the fluorescent antigen-antibody technique; this is a particularly valuable technique for precise localization of the virus in the infected fœtus.

(*e*) Virus can also be demonstrated by electron microscopy.

(2) *Rubella Antibody*

The virus neutralization (VN) test remains the most important method of measuring protective antibody, but there are certain technical drawbacks which limit its use and the tests are time consuming. The hæmagglutination-inhibition (HI) antibody test described by Stewart *et al.* (1967) has been used on an extensive scale in recent years and has yielded information of great significance to our understanding of this problem. With a few exceptions, very good correlation is found between the VN and HI tests.

(3) *Other Immunological Studies*

(*a*) *Immunoglobulin estimations:* The immunological response of the fœtus to intrauterine rubella is also reflected in the increased amount of IgM found in cord sera at birth and in the early months of life. Immunoglobulin levels of IgG, M and A can be measured by the single gel diffusion technique modified from Mancini *et al.* (1965). In our studies, reported from the Hospital for Sick Children, Great Ormond Street, in conjunction with the Department of Immunology, values are expressed as a percentage of the 'Proposed British Research Standards for Immunoglobulins G, A and M' (Dudgeon *et al.* 1969).

(*b*) *Lymphocyte transformation:* As a measure to determine cellular immune function the response of peripheral blood lymphocytes to stimulation by phytohæmagglutinin can be used (Marshall *et al.* 1970).

CLINICAL MANIFESTATIONS

The original clinical descriptions of congenital rubella laid emphasis on cataracts, hearing defects and congenital heart disease with or without mental retardation. These were usually referred to as the rubella syndrome. Subsequent detailed studies of several hundred cases following the 1963–5 epidemics have shown that intrauterine rubella can frequently lead to a multisystem disease in which many organs may be affected (*American Journal of Diseases of Children*, 1965, 1969, Cooper *et al.* 1969, Dudgeon *et al.* 1969).

Table 2

Main clinical manifestations of congenital rubella

(1) Eye	Cataracts, often bilateral. Retinopathy. Microphthalmos. Glaucoma; cloudy cornea
(2) Heart and blood vessels	Patent ductus arteriosus. Pulmonary stenosis. Ventricular septal defect. Myocardial damage. Peripheral artery stenosis
(3) Ear	Perceptive or sensorineural deafness
(4) Central nervous system	Mental retardation. Microcephaly, full fontanelle. Cerebral palsy. Meningoencephalitis
(5) Visceral and hæmatological	Hepatosplenomegaly, hepatitis. Thrombocytopenic purpura. Jaundice, anæmia, generalized adenopathy
(6) General and miscellaneous	Low birth weight. Failure to thrive, short stature. Osteopathy. Hypogammaglobulinæmia. Increased susceptibility to infection. Abnormal dermatoglyphics. Chronic rubella type rash

The more important clinical manifestations are shown in Table 2, together with the approximate incidence of these defects in congenital rubella. What Table 2 does not show, and what is most important from the point of view of management of these children, is that the majority of cases have

multiple defects. Our own studies in 1965 showed that 46% had more than one defect (Dudgeon 1965), but more recent data suggest that the figure is of the order of 75%. Deafness is the only defect that may occur by itself.

THE PATHOGENESIS OF RUBELLA

Under this heading consideration has to be given to the factors determining the risk of infection to the fœtus and the way in which virus spreads from mother to fœtus. The two principal factors underlying the vulnerability of the fœtus are the maternal immune status and the gestational age at which infection occurs. There is both clinical and epidemiological evidence to support the view that congenital rubella follows primary rubella in the susceptible pregnant woman and this may occur after either clinical or subclinical disease. This has since been confirmed by virological studies. Although subclinical reinfection occurs in rubella, probably more often than is supposed, there is no evidence that reinfection or 'second' attacks of rubella lead to fœtal damage.

The vast majority of cases of congenital malformations are associated with maternal rubella in the first trimester, but the risk extends beyond this stage without a sudden cut-off at the twelfth week. Accurate estimates of rubella defects are extremely difficult to obtain. The crude figures show a very much higher incidence in the first and second months of pregnancy than in the third, but a number of cases of hearing defect still occur between the 12th and 16th weeks. A recent study from Hardy and her associates (1969) at the Johns Hopkins Hospital, Baltimore, indicates that there may be a risk of fœtal damage after the first trimester. In a prospective study during the 1964–5 pandemic they encountered 24 cases in which there was clinical and virological evidence of rubella between the 14th and 31st weeks of pregnancy; in 19 of these it was after the 16th week. The anomalies were somewhat different from those generally observed in congenital rubella. There were no visual defects and 2 cases of peripheral pulmonary stenosis. Several had hearing and language or communication disorders. Further studies are needed to assess the significance of these findings, but it is clear that more information is needed on the effect of rubella at any stage in pregnancy.

Whatever figures on the incidence of congenital malformations are looked at there is a lack of correlation between the incidence of malformations on the one hand and the recovery of virus from the fœtuses on the other. Virus can be recovered from close on 100% of aborted fœtuses using the trypsinized cell culture technique, but even at this period of maximum susceptibility the incidence of defects is never as high (Dudgeon 1969). This indicates that under certain conditions the fœtus can overcome the infection. Virus studies point to this, as do twin studies in which it has been shown that one twin may be severely damaged and the other apparently normal, though with serological evidence of intrauterine infection.

Mode of Spread

From the point of view of risk to the fœtus the most important stage in the pathogenesis of rubella is the maternal viræmia which may occur up to seven days before the time of onset of the rash. It is probable that by the time the rash itself has appeared, or at an equivalent time in subclinical cases, the virus has already reached the placenta if not the fœtus.

Virological studies have revealed that rubella virus can be recovered from the placenta and from most fœtal tissues after therapeutic abortion. Moreover virus can be recovered from about 80–90% of cases at birth and from approximately 10% at one year of life. Chronic infection may persist for long periods in certain situations such as the inner ear and cataract tissue.

Pathology of Congenital Rubella

The next point to consider is how the damage is caused once the virus has reached the fœtus. Few of the lesions which have been described show any specific cellular changes such as inclusion body formation, such as are found in fœtal varicella or vaccinia. There is a marked absence of necrosis and even more so of inflammatory changes in congenital rubella. We have then to consider whether the changes are caused by a direct virus action on growing cells – we know that the virus is there because it can be isolated – or by interference with the blood supply, or both.

The Placenta

Studies by Töndury & Smith (1966) and by Driscoll (1969) have revealed important changes in the placenta with particular emphasis on vascular damage and focal necrosis of the trophoblasts. Angiopathy in chorionic and fœtal tissues and of progressive sclerosing villous inflammation is a frequent finding. The fact that this may occur at such an important stage in the vascularization of the fœtus could lead to serious interruptions in the blood supply to vital organs. Töndury & Smith (1966) have also demonstrated severe damage to

the endothelial cells in the capillaries and vessels of the chorion. In their opinion much of the damage is caused by small emboli of infected cells.

Compared with fœtal and perinatal infection caused by other viruses such as variola-vaccinia, varicella, herpes simplex and Coxsackie B, where tissue destruction is often severe, the pathological changes in congenital rubella are not very marked. The most obvious gross changes are general retardation in fœtal growth and the small size of certain organs, notably the brain, eye, adrenals and sometimes the thymus. The main histopathological lesion seen is endothelial necrobiosis affecting the small fœtal blood vessels, for example in the brain and myocardium. Another striking feature is the virtual absence of any inflammatory response (Töndury & Smith 1966) and the same absence of histopathological changes is found in tissues from which virus can be isolated (Woods *et al.* 1966).

In seeking for an explanation of the mechanisms responsible for fœtal damage in congenital rubella it must be emphasized that the evidence available at the present time is strictly limited. There is a lack of detailed pathological data on human material, and the lack of a suitable animal model makes it difficult to study these processes experimentally. There appear to be three possible mechanisms which might be implicated in the production of fœtal damage:

(*a*) *Inhibition of cell growth:* Retardation or even inhibition of cell growth could result directly from virus multiplication or be due to damage to chromosomes. Another mechanism which may be involved is the effect of an inhibitor which Plotkin & Vaheri (1967) demonstrated in diploid cell cultures infected with rubella virus. Experimental studies by Plotkin *et al.* (1965) in diploid cells derived from human fœtal kidney and lung showed that cell division was slowed, followed by complete mitotic arrest. In contrast, cultures derived from skin and pharyngeal mucosa and infected with virus became chronically infected without evidence of inhibition of cell growth. Cytogenetic studies of chronically infected cultures showed a high incidence of chromosomal breaks and the same observations concerning chromosomal breaks have been made on cell cultures derived from fœtuses in which pregnancy was terminated on the grounds of exposure to maternal rubella, but only in those from whom virus was isolated (Boué & Boué 1969). Although it would be unwise to try to draw exact parallels between *in vitro* experiments and what occurs *in vivo*, it is possible that the retardation of cell growth with loss of viability results partly from chromosomal damage and partly from some inhibitory factor such as described by Plotkin & Vaheri (1967).

(*b*) *Cytolytic action:* As already stated, cell necrosis occurs in congenital rubella, but is is not a marked feature; neither does the virus produce much evidence of cytolytic action in cell cultures.

(*c*) *Interference with the blood supply:* The effect on the endothelium of small blood vessels is a striking feature in congenital rubella. As much of the damage is caused at a time when many vital organs are developing, as is the vascular supply of the fœtus as a whole, it is reasonable to assume that any interference with the blood supply could lead to fœtal damage of varying severity.

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