

# Teratogen Update: Congenital Rubella

WILLIAM S. WEBSTER

*Department of Anatomy and Histology, University of Sydney, Sydney, Australia*

For teratologists, rubella does not seem to invoke the fascination of thalidomide despite the fact that in a single epidemic in the United States it caused more birth defects in one year (Cooper, '68) than thalidomide did during its entire time on the world market (Schardain, '93). Surprisingly little is known about the pathogenesis of rubella-induced birth defects, although it produces a spectrum of defects as distinctive as those produced by any other teratogen.

In this review and update an attempt has been made to examine rubella in the wider context of human teratogens with respect to its discovery and the pathogenesis of the virus-induced congenital malformations and late manifestations. The extensive clinical manifestations of congenital rubella are well known and are the subject of many reviews and chapters in textbooks (e.g., Hanshaw et al., '85; South and Sever, '85; Preblud and Alford, '90; Gilbert, '91; Best and Banatvala, '95), and this aspect is not specifically covered in this update.

## HISTORICAL ASPECTS

In 1940, Australia had a severe rubella epidemic. There was debate at the time that it might be due to a mutated form of the virus, which showed particular virulence, since many adults were infected and there was a high incidence of associated arthritis and arthralgia. However, the generally accepted scenario is that because Australia was involved in the second world war, many country people had come to Sydney and into crowded army camps as part of the war effort, and because of their previous isolation they had not been exposed to earlier outbreaks of rubella. Hence, when the epidemic struck, they presented as a large pool of susceptible adults. The infection was further spread by soldiers going home on leave (Burgess, '91).

In 1941, Norman Gregg (Fig. 1), Senior Ophthalmic Surgeon at the Royal Alexandra Hospital for Children in Sydney, reported that in the first 6 months of the year he had seen 13 babies with bilateral congenital cataracts, and his immediate colleagues had seen a further 7 cases. He contacted colleagues around Australia and eventually recorded a total of 78 cases. Many of the babies had difficulty in feeding and failure to thrive and had symptoms (such as difficulty in taking the breast) that were indicative of heart defects.

The cataracts were considered unusual since they involved all but the outmost layers of the lens (Fig. 2). Gregg suggested that this implied that the cataractous process had begun early in the life of the embryo (Gregg, '41). He wrote: "Although one was struck with

the unusual appearance of the cataracts in the first few cases, it was only when similar cases continued to appear that serious thought was given to causation. The remarkable similarity of the opacities in the lens, the frequency of an accompanying affection of the heart and the widespread geographical incidence of the cases suggested there was some common factor in the production of the diseased condition, and suggested it was the result of some constitutional condition of toxic or infective nature rather than of a purely development defect" (Gregg, '41). This latter speculation was perhaps inspired by the chapter on congenital anomalies in Duke-Elder's *Text Book of Ophthalmology* ('38), in which it is stated: "Thus cataract may be produced in young animals by feeding the mother on naphthalene or by exposure to X-rays. It is conceivable that toxic or infective processes in the mother may cause a derangement in the lens of the fetus. . . ."

Naturally, Gregg was concerned about this "outbreak" of cataracts and asked parents about their child's history. Eventually he heard two parents talking about the rubella they had in early pregnancy, and he made the link (Burgess, '91). Gregg again consulted with his colleagues to check for a history of rubella in their cases, the association was confirmed. In 68 of the 78 cases there was a definite history of rubella in early pregnancy. In October 1941 he gave a talk to the Ophthalmological Society of Australia in which he stated that "maternal rubella infection in early pregnancy was the cause of the babies' defects" (Gregg, '41). This included not only the cataracts, but also congenital heart disease. Fifteen of the infants had died by the time the paper was presented, and the autopsies had shown a variety of heart defects including the frequently observed patent ductus arteriosus (Burgess, '91).

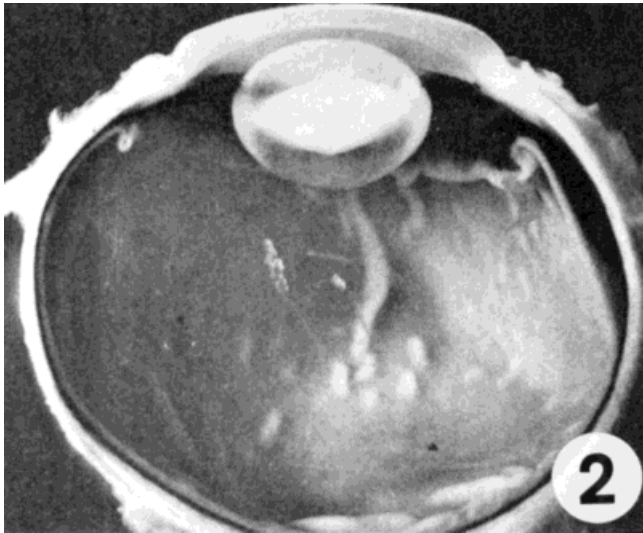
To fully appreciate Gregg's achievement in making the association between maternal rubella and congenital cataracts, it is necessary to consider the medical priorities and positions of the day. Medicine was still dominated by infectious diseases. In Australia in 1940-1941, in addition to the epidemic of rubella, there were also epidemics of whooping cough, diphtheria, and the most widespread epidemic of meningitis ever. Although there were sulfonamide drugs, it was before the availability of penicillin; and many deaths were associated

\*Correspondence to: Professor W.S. Webster, Department of Anatomy and Histology, University of Sydney, Sydney, N.S.W. 2006, Australia.

Received 16 February 1998; Accepted 9 March 1998



**Fig. 1.** Sir Norman McAlister Gregg portrait by Sir William Dargie, photographed by Raymond de Berquelle, with kind permission of Miss Sheila Gregg.



**Fig. 2.** An opaque nuclear cataract from an infant with CRS who died at 3 weeks of age. From Boniuk and Zimmerman ('67) *Arch Ophthalmol* 77:455–473. Copyright 1967–73 American Medical Association.

with infectious diseases, particularly in children. Eighty children died from whooping cough in the main children's hospital in Sydney in 1940. In contrast, rubella is only a mild disease in adults with coldlike symptoms lasting 1–7 days followed by a fine, pinpoint nonitchy rash which lasts 3–4 days. Rubella was considered a nuisance—nothing more.

Despite the ideas presented by Duke-Elder ('38), it was widely believed that birth defects were inherited. To quote Warkany ('73), "In the 1930s the medical literature was replete with statements that all congenital malformations are inherited; the term 'congenital' became synonymous with 'hereditary.' There were times

when it was considered unscientific to invoke non-genetic factors." It is remarkable that Gregg made the association despite these prejudices and beliefs of the time.

It is perhaps significant that prenatal exposure to rubella causes cataracts—malformations or disruptions that are not exclusively prenatal in origin. An ophthalmologist like Gregg would have been very familiar with the range of cataracts that develop in the adult. He might also have been aware of experiments which had shown that cataracts could be caused by administration of naphthalene to adult rabbits (Bouchard and Charrin, 1886) or to pregnant rabbits (van der Hoeve, '13). This latter experiment was referenced by Duke-Elder ('38), and the concept of transplacental passage of toxins, drugs, and exanthems was extensively discussed in the other important ophthalmological text of the time (Mann, '37). It was also known that the syphilis spirochaete could infect the fetus and could even cause ocular defects (Mann '37; Duke-Elder, '38). Hence, as an ophthalmologist, Gregg was well positioned to postulate a nongenetic cause for these cataracts of prenatal origin.

Perhaps as a reflection of the high esteem in which Dr. Gregg was held in Australia, the *Medical Journal of Australia* carried an editorial on Gregg's talk 7 weeks after his presentation and before his paper was published (Editorial, '41). Although generally accepting the concept, the editorial urged caution, stating: "the association is not entirely proven." There was also the comment that "many persons will at once ask themselves whether the use of sulphanilamide in the treatment of infected mothers may not have had a bearing on the formation of cataract in the fetus." Considering this was almost 20 years prior to thalidomide, many teratologists may find this suggestion unexpected. The editorialist contacted Gregg about this idea and in a personal communication he stated that "in no case in the series of 78 cases was a sulphanilamide compound exhibited" (Editorial, '41).

The National Health and Research Council of Australia also responded to Gregg's findings, and in September 1942 a South Australian medical researcher, Dr. Swan, was appointed to enquire further. Letters were sent to all general practitioners in the state of South Australia informing them of Gregg's findings and asking them to complete a form for all children born to women who had an acute exanthema during pregnancy. The study showed that there were 49 cases where the mother had been exposed to rubella during pregnancy, with 31 having congenital malformations including cataract, deaf mutism, heart disease, microcephaly, and mental retardation. In all but 2 of the 31 cases, rubella had been contracted in the first 3 months of pregnancy (Swan et al., '43). Deafness was now recognised as a major effect of rubella in pregnancy, and an article in a Sydney newspaper in 1945 stated that there were more than 140 deaf children in the state of New South Wales whose mothers suffered from German measles in the

1940 epidemic. Swan also suggested that the type of congenital malformation was dependent upon the stage of pregnancy at which the mother acquired rubella.

It was several years before editors of medical journals outside Australia made comment on Gregg's ideas. Despite the extent of the evidence, a *Lancet* editorial in 1944 (Editorial, '44) was almost contemptuous of the findings, "... but though the possibility remains he cannot yet be said to have proved his case." The editorial concluded that "the lay public have always held that congenital malformations have an extrinsic explanation—from being frightened by a dog to falling down stairs—and it will be strange if the influence of a mild illness in the first months of pregnancy, accompanied by a rash, has escaped attention."

An editorial in the *British Medical Journal* (Editorial, '45) was less critical and referred to similar findings in America and England, and suggested that the association with congenital malformations seems to be a new manifestation of rubella since it was unlikely that such a dramatic sequence could have been overlooked in the past. However, it was still concluded "we cannot exclude the possibility of a chance association between congenital defects and true maternal rubella."

Following the publication of a number of case reports, the response in the American literature was more positive. An editorial in the *Journal of the American Medical Association* (Editorial, '46) seemed to accept the relationship with the suggestion that it was due to a new capacity of the rubella virus from Australia. The article concluded that "special efforts must be made to prevent rubella in early pregnancy" (by avoiding contact with infected people).

The Australian Nobel laureate, Sir Macfarlane Burnet, was Gregg's strongest advocate. He informed colleagues in the U.S., including Professor Conrad Wesselhoef, Clinical Professor of Infectious Diseases at Harvard School of Public Health and Boston University School of Medicine (Lancaster, '92). Professor Wesselhoef wrote a review of rubella for the *New England Journal of Medicine* (Wesselhoef, '47a,b), and in this rather entertaining review the teratogenic effects of rubella were unequivocally accepted.

Hence, like so many other human teratogens, the teratogenic effects of rubella were discovered by a clinician who observed a cluster of congenital defects and pursued the possibility of a common etiology. The subsequent development of a vaccine was too late for epidemics in Europe in 1963, and in the United States in 1964–1965 during which an estimated 1.8 million people were infected and 30,000 children born with rubella-associated birth defects (Cooper, '68).

Vaccination programmes have now led to a dramatic drop in the incidence of congenital rubella. Vaccination does not completely eliminate the problem of congenital rubella for an individual, since a low percentage of those vaccinated show no or very low immune response, and even in successful vaccination the duration of protection can be very variable (Braun et al., '94).

Rubella vaccine is a live attenuated virus which causes a mild and benign infection with minimal shedding of the virus; although some individuals can develop chronic symptoms following vaccination (Howson et al., '92). Since the vaccine virus can cross the placenta, some concern has been expressed that there may be a risk if vaccination occurs during pregnancy or immediately before pregnancy (Centers for Disease Control, '89)—but this risk is now thought to be negligible (Burgess, '90).

## CONSEQUENCES OF MATERNAL INFECTION DURING PREGNANCY

### Incidence of fetal infection

The term *fetus* is used in this article to describe the conceptus at any stage of gestation. The risk of fetal infection varies according to the time of onset of maternal infection. In a prospective study of pregnant women with confirmed rubella, fetal infection was indicated by IgM antibody soon after birth or persistence of IgG after the first year. Fetal infection occurred in 81% (13/16) of infants exposed in the first trimester [0–12 weeks based on last menstrual period (LMP)]. The 3 infants that escaped infection were 2 exposed at 12 weeks and 1 whose mother's rash was 7 days after her LMP. This latter case supports the concept that rubella infection before the last LMP presents a negligible risk to the embryo (Enders et al., '88). In the second trimester the fetal infection rate decreased from 67% (12/18) at 13–14 weeks to 25% (8/32) at 23 to 26 weeks; and in the third trimester the infection rates were 35% (11/31) for weeks 27–30, 60% for weeks 31–36 (15/25), and 100% in 8 infants exposed >36 weeks (Miller et al., '82). The reasons for the variations in placental transfer with gestational age are unknown. It is possible that changes in placental structure could lead to the increased resistance in the second trimester, with the later thinning of the trophoblast permitting late gestation passage (Kaplan, '93).

### Types of birth defects

The range of defects caused by rubella infection has been well documented (e.g., Hanshaw et al., '85; Best and Banatvala, '95), and they almost exclusively result from infection in the first 16 weeks of gestation. Infection in the fifth month or later does not usually cause disability, although cases of deafness have been reported after infection as late as 28 weeks and peripheral pulmonary artery stenosis as late as 24 weeks (South and Sever, '85), and there may be growth retardation associated with third trimester infection (Miller et al., '82). The extent of spontaneous abortion induced by rubella has not been determined but may not be particularly high (Lundström, '62). The main defects associated with rubella infection are deafness, eye defects such as cataracts, cardiovascular defects, particularly patent ductus arteriosus, and CNS damage leading to mental retardation. The term *congenital*

*rubella syndrome* (CRS) is used, as defined by South and Sever ('85), to denote any combination of the findings known to result from gestational rubella.

### Neonatal manifestations

Once the virus has entered the fetus, it remains for the rest of gestation. Associated with this continuing infection the affected infants may show a wide range of transient symptoms at birth (Best and Banatvala, '95). Usually infants with these transient findings have growth retardation and may fail to thrive.

### Postnatal development of defects

Monitoring of CRS survivors for up to 50 years (McIntosh and Menser, '92) has revealed that they show a number of late-onset manifestations (Menser et al., '67a, '69, '74; Forrest et al., '69a, '71) including insulin-dependent diabetes (~50 times the rate in the general population), thyroid dysfunction, and a rare neurodegenerative disorder—panencephalitis. These conditions may result from prenatal damage, damage from ongoing viral infection, immune-mediated cell destruction, and autoimmune response triggered by molecular mimicry (Rawls, '74; Sever et al., '85; Wolinsky, '90; Frey, '94).

### FETAL IMMUNE RESPONSE TO RUBELLA

Prior to the development of the maternal immune response, the rubella virus spreads through the blood and may infect multiple maternal tissues, including the placenta. Maternal antibody production subsequently results in the disappearance of the virus from the blood but it may persist for months in the placenta. Probably as a result of placental damage (Töndury and Smith, '66), the virus frequently crosses the placenta and infects the fetus (Alford et al., '64).

Once the fetus is infected it appears to be unable to rid itself of the virus; this applies whether infection takes place early or late in gestation. For at least the first trimester, the fetus is incapable of making a normal immune response as occurs in the mother. Instead, it must rely on transfer across the placenta of maternal IgG antibodies. This process starts in the first half of gestation, with rubella-specific IgG detected in coelomic fluid at 6 weeks gestation (Jauniaux et al., '95); but placental transfer appears to be inefficient at this stage, as levels in the fetal blood by midgestation are only 5 to 10% of those in maternal serum. Transfer increases progressively throughout gestation and cord maternal IgG levels at term may exceed those of the mother (Preblud and Alford, '90). Hence, in the first trimester, the fetus appears to be relatively defenseless against the virus and most damage occurs after infection during this period.

In the second trimester the risk of fetal infection decreases significantly; this is thought to be due to changes in the placenta rather than an enhanced fetal immune response, since fetal infection still takes place later in gestation when the fetal immune response is well developed. As indicated above, fetal damage is rare

if fetal infection occurs after about 16 weeks gestation. This implies that at this stage, the combined fetal response and transfer of maternal antibodies is sufficient to limit viral activity. The fetus starts to produce its own antibodies at about this time. Although pre-B cell lymphocytes can be identified as early as 7–8 weeks gestation in the fetal liver, the immunoglobulin-secreting plasma cells appear later with fetal IgM detectable at 15 weeks gestation and IgG and IgA at 20 and 30 weeks, respectively (Gitlin and Biasucci, '69). Infants with congenital rubella infection may have adult levels of rubella-specific fetal IgM and elevated levels of fetal IgG and IgA at birth (Alford et al., '67; Enders, '85), as well as high levels of maternally derived IgG. The interaction of antibodies with complement is probably reduced compared with adults since the concentration of complement components increases slowly from midgestation to about 50% of adult values by birth (Notarangelo et al., '84; Winkelstein, '92).

It has been suggested that once the virus has entered embryonic cells, it is transferred to their progeny during cell proliferation, resulting in infected clones of cells (Rawls et al., '68; Woods et al., '66). In this way, it avoids the maternal and fetal antibodies. The capacity to attack viral-infected cells involves T lymphocytes, natural killer cells, mononuclear phagocytes, and interferon production. Lymphocyte precursors first migrate to the thymus at about 7 weeks gestation (Royo et al., '87), and T cell lymphocytes have essentially adult expression by 17 weeks gestation (Lobach et al., '85). In vitro studies suggest that the T cells are functional by 12 weeks gestation or earlier (Durandy et al., '82; Yarchoan and Nelson, '83). However, overall T cell reactivity in the fetus is impaired compared to adults with diminished production of certain lymphokines (Wilson, '90). Natural killer lymphocytes have been detected in the liver by midgestation, and levels in the blood of neonates and adults are similar (Ueno et al., '85). Alpha-interferon is readily detectable in the sera of midgestational rubella-infected fetuses (Lebon et al., '85) and may be present as early as 7 weeks gestation (Preblud and Alford, '90). This may represent the earliest embryonic response to rubella infection. In vitro studies indicate rubella virus replication is limited by interferon, but the virus can survive in interferon-producing cells (Frey, '94).

Hence, from about midgestation the fetus progressively has the capacity to launch both a humeral and cytotoxic response to the virus. Together with the transferred maternal IgG, the fetus can largely protect itself from viral damage for the remainder of gestation—although it is incapable of getting rid of the virus. It is possible that the immunological response of the fetus to rubella is handicapped by infection of the primitive immune cells and interference with their proliferation (South and Sever, '85), and some CRS infants appear to have permanent immune damage (although they do not develop immunological tolerance to rubella).

### **PATHOGENESIS OF FETAL DEFECTS**

The particular pattern of malformations in congenital rubella is unlike that seen with any known chemical teratogen in either humans or experimental animals. Many of the defects are at the interface of malformations and disruptions. For instance, both cataracts and deafness may result from damage to established structures (e.g., formed primary lens fibres or the cochlear duct) rather than interference with organogenesis of the eye or inner ear. Most organogenic processes appear to be unaffected by the rubella virus, including closure of the neural tube, formation of the upper lip and palate and limb development.

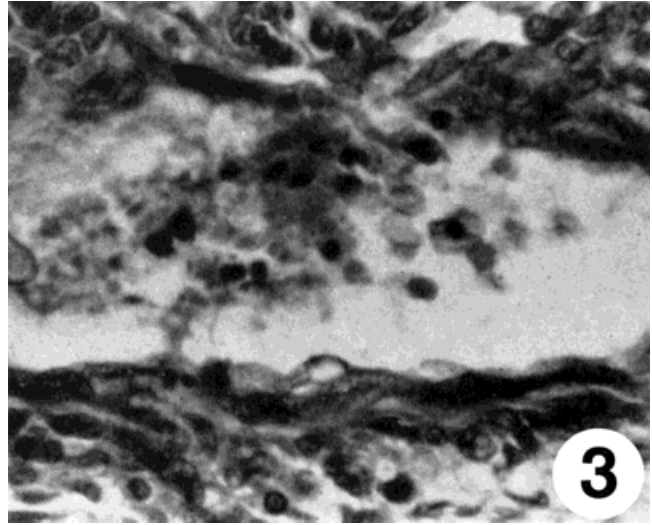
#### **Animal models**

It is usual to try to study human teratogens by establishing animal models, and there have been many attempts to develop animal models of CRS. Low incidences of cataracts have been reported in the offspring of infected pregnant rats (Cotlier et al., '68), rabbits (Kono et al., '69; Machado et al., '76), and monkeys (Delahunt and Rieser, '67). However, other studies with pregnant rats (Avila et al., '72), mice (Menser et al., '78; Rayfield et al., '86), rabbits (London et al., '70; Menser et al., '78), ferrets (Rorke et al., '68; Fabiyi et al., '67; Menser et al., '78), and monkeys (Parkman et al., '65; Sever et al., '66; Amstey, '69; Patterson et al., '73) have not shown these defects. Abnormalities of the heart (Cotlier et al., '68; Amstey, '69), cerebral blood vessels (Rorke et al., '68), and inner ear (Delahunt and Rieser, '67) have been reported in some studies, but incidences are very low and inconsistency has limited their usefulness. With respect to the late manifestations of CRS, two studies have reported pancreatic changes suggestive of diabetes (Menser et al., '78; Rayfield et al., '86).

Hence, unlike the situation for most human teratogens, animal models of CRS are not particularly useful and have not contributed much to the understanding of the pathogenesis of the defects. However, in contrast to the situation for other human teratogens, there is good histopathology of infected abortuses, and these have provided valuable information on the pathogenesis of the abnormalities.

#### **Studies of CRS pathology**

The major pathological study was carried out on 57 therapeutic abortuses obtained in good condition, mostly after elective abortion, following the clinical diagnosis of maternal rubella during the first trimester of pregnancy (Töndury and Smith, '66). The average time from maternal rubella infection until therapeutic abortion was 33 days. Most of the fetuses were from the first trimester and comparisons were made with apparently normal fetuses of similar age. None of the rubella-exposed fetuses had gross external malformations, but 68% had microscopic abnormality. Much of the information on pathogenesis described below is based on this study.



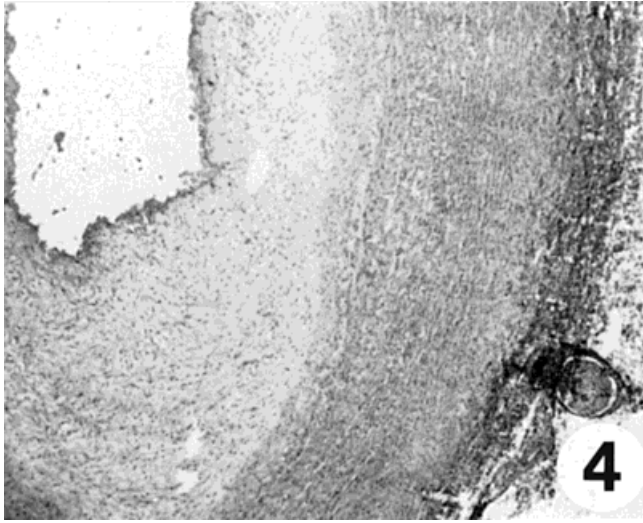
**Fig. 3.** Destruction of a part of the carotid artery wall with desquamation of necrotic cells into its lumen. From an abortus 43 days fetal age, 10 days postmaternal rubella. From Töndury and Smith ('66) *J. Pediatr* 68:867-879. Copyright Alan R. Liss, Inc.

#### **Placental involvement**

During the period of maternal viremia the placenta may become infected. Although the virus may persist for months in the placenta, recovery of the virus from the placenta at birth is infrequent (Catalano et al., '71). In the Töndury and Smith study ('66) the chorion was obtained from 12 specimens 10 to 45 days after the occurrence of maternal rubella. Eight of the specimens showed necrotic foci in the epithelium of the chorionic villi and in the endothelium of the capillaries and larger vessels. Damaged endothelial cells were observed to be desquamated into the lumen of the vessels, and on this basis it was proposed that the virus entered the fetal circulation by embolic transport. Similar damage to villous capillaries have been seen in other studies of placentas from therapeutic abortions associated with maternal rubella (Driscoll, '69; Ornoy et al., '73). Infection at later stages of pregnancy causes multifocal chronic mononuclear cell infiltrates in the placental membranes, cord, and decidua, along with vasculitis; these culminate in placental hypoplasia and placentitis (Garcia et al., '85). These changes are not specific for rubella but may be caused by herpes or cytomegalovirus.

#### **Heart defects**

Once the virus has entered the early embryo, a chronic nonlytic infection is established (Plotkin, '75) and the virus can infect virtually any organ. Detectable virus was found in most organs of the body of 2 congenital rubella infants that died in the neonatal period (Bellanti et al., '65). Presumably, spread of the virus is initially through the vascular system, and many of the first trimester abortuses examined by Töndury and Smith ('66) showed noninflammatory damage to the endothelial cells lining blood vessels (Fig. 3)



**Fig. 4.** Left pulmonary artery showing extreme intimal proliferation from an infant with CRS who died at 6 months age  $\times 30$ . From Esterly and Oppenheimer ('67) *Arch Otolaryngol* 98:246–248. Copyright Waverly, Williams and Wilkins.

and the heart. Thirty-nine percent of the embryos showed necrotic cellular damage in the myocardium, especially in the subendocardial cells of the left atrium, with 16% showing impaired development of the septum secundum; and 2 cases showed delayed closure of the membranous ventricular septum. Direct viral damage of the septa of the heart may be the cause of the increased incidence of septal defects associated with first trimester infection. Damage to endothelial cells can also lead to thrombosis of small vessels and surrounding tissue necrosis (Plotkin, '75). Increased hemosiderin was noted in several specimens and was interpreted as evidence of hemorrhage. Similar endothelial necrosis and focal myocardial degeneration without inflammatory reaction was seen in some abortuses examined by Driscoll ('69). The absence of any inflammatory reaction in the infected fetal tissues in the first trimester is characteristic (Dudgeon, '69).

Cardiac malformations occur after infection at any time in the first 12 weeks of gestation but are rare after this time (South and Sever, '85). The most common cardiovascular lesions are patent ductus arteriosus associated with infection 11 to 48 days after fertilisation, and stenosis of the pulmonary artery and its branches 16 to 57 days after fertilisation (Ueda et al., '79).

First trimester infection and viral damage to the lining of the developing heart and blood vessels may be further aggravated by continuing intracellular infection and immunological response during the rest of gestation. In autopsied infants with CRS, common findings are generalised fibromuscular proliferation of the arterial intima of large- and medium-sized arteries in both systemic and pulmonary circulations (Fig. 4)—in some cases severe enough to be occlusive (Singer et al., '67; Forrest et al., '69b; Esterly and Oppenheimer, '67,

'73; Menser and Reye, '74). Conversely, in 3 neonatal deaths following first trimester maternal rubella, the normally muscular ductus arteriosus was described as having a large lumen and thin wall. There was moderate replacement of muscle and occasionally elastic tissue with collagen. The internal elastic lamina was either absent or ill-defined (Swan, '44) and was the probable cause of the failure to close.

Infection starting later in gestation is controlled by the combined fetal immune response and transferred maternal IgG, and this appears to limit vascular damage to such an extent that there is no associated organ damage.

### Eye defects

Opacities in the primary lens fibres resulting in a characteristic central or nuclear cataract (Fig. 2) were the unique symptoms observed by Gregg. He correctly reasoned that involvement of the central lens fibres implied that the cataractous process had begun early in the life of the embryo. The period of susceptibility for rubella-induced cataracts is relatively short and has implications for the pathogenesis. In one study of CRS infants, 13 babies had cataracts following the onset of the maternal rash between 12 and 43 days after fertilisation, while 33 fetuses infected after 43 days did not develop cataracts (Ueda et al., '79).

Lenses from first trimester rubella-infected abortuses showed pyknotic nuclei, cytoplasmic vacuoles, and inclusion bodies in the primary lens cells and retardation of lens development (Töndury and Smith, '66; Cordes and Barber, '66). Late changes included degeneration of some primary lens fibres and evidence of active disease in the newly developing equatorial lens fibre cells, indicating chronic infection. Despite infection with the virus, the equatorial cells appear to retain their ability to divide since the lens at birth in CRS is many times the size at the time of infection (Gray, '60). Examination of the cataractous eyes in neonates showed that the nuclear portion of the lens was often necrotic, while some of the more peripheral fibres had disintegrated and were replaced by vacuoles (Swan, '44; Boniuk and Zimmerman, '67; Kresky and Nauheim, '67). Retention of nuclei in surviving lens fibres appears to be characteristic of rubella cataracts.

In an attempt to explain the restricted period of susceptibility for cataract formation, it was proposed that the virus does not reach the lens through the blood supply but from the amniotic fluid (Karkinen-Jääskeläinen et al., '75). According to this hypothesis, the virus could only gain access to the lens as long as the invagination and detachment of the lens vesicle from the surface ectoderm was incomplete. However, the lens pit closes at about 33 days gestation (O'Rahilly, '83), which is rather early for the known period of cataract susceptibility.

Evidence supporting this hypothesis was provided by a study in which eye rudiments from therapeutic abortions were cultured in media containing rubella virus, and viral antigens were detected using immunofluorescence meth-

ods (Karkinen-Jääskeläinen et al., '75). Lenses infected in the open-lens-vesicle stage developed normally at first, but after about 10 days the differentiating lens fibres underwent vacuolar degeneration: first in the equatorial zone where terminal mitosis takes place. Specific fluorescence was detected in the lens and surrounding tissues. Lenses infected after closure of the lens vesicle showed no sign of damage and could be maintained for up to 4 weeks. The investigators showed that the lens itself had not become resistant to the virus by culturing lenses in the closed stage after excision of the surrounding lens capsule. In this case the entire lens became infected and showed degeneration and fluorescence.

Although these experiments were used to support the idea that infection took place from the amniotic fluid, such an explanation is not necessary. It is possible that the virus enters the developing lens after damaging the walls of the blood vessels supplying the eye. The hyaloid artery provides a network of blood vessels around the developing lens as early the fourth week of gestation. This network continues to increase in complexity and remains in the optic cup until regression of the hyaloid artery in the third trimester (Barber, '55). However, the lens becomes increasingly separated from this blood supply by the development of a lens capsule. This derivative of the basement membrane of the lens epithelium begins to form at the end of the fifth week and gradually increases in thickness. It is possible that prior to development of the capsule, the virus can enter the developing lens from the surrounding blood vessels; as the capsule thickens it increasingly protects the lens from infection (Zimmerman, '65). If the virus is already in the lens tissue at the time of capsule development, the capsule may have the effect of isolating the lens from any immunological response, and the virus can continue to replicate and build up to high concentrations observed in congenitally cataractous lenses. The virus can persist in the cataractous lens for up to 3 years postnatally and may continue to damage the lens (Menser et al., '67b).

In some CRS cases there is damage to other ocular structures, including focal necrosis of the pigment epithelium of the retina and necrosis of the ciliary body and iris as well as microphthalmia (Givens et al., '93). Retinopathy and glaucoma (Boniuk and Zimmerman '67) occur after infection over a much longer period (2–117 days gestation) than do cataracts (Ueda et al., '79); and this again may be due to the virus entering the optic cup via damaged hyaloid blood vessels. The retina and other parts of the optic cup are not protected by a capsule and may remain vulnerable until spread of the virus through the blood supply is controlled by development of the immune system.

Hence, it is clear that the rubella virus is cytopathic to some of the lens fibres in the developing eye causing degeneration of the primary lens fibres and damaging the newly formed lens fibres, resulting in a whitish haze toward the lateral borders of the lens (Töndury and Smith, '66). The virus appears to be able to produce focal necrosis in most structures of the eye, but it has its predominant effect on the lens—perhaps as a consequence of its subsequent isolation by the lens capsule.



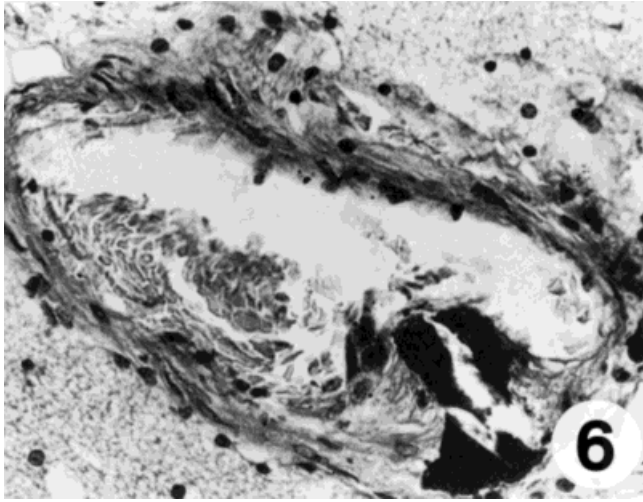
**Fig. 5.** Basilar membrane of the basal turn of the left cochlear duct from a 53-mm abortus. There is lysis of the cytoplasm of the epithelium of Corti's organ and loss of cells. The mother developed rubella on the 37th day of pregnancy. This figure was first published in the *BMJ Gray* ('60) *BMJ*:1388–92 and is reproduced by permission of the *BMJ*.

### Deafness

Sensorineural deafness, which can progress after birth, is the most common rubella-associated defect and mainly results when rubella infection occurs in the first 16 weeks of gestation. In one series, it occurred in almost every case when infection took place in this period (Ueda et al., '79); vestibular function is rarely impaired. As is the case with the lens vesicle, the otic vesicle is surrounded by a basement membrane and a capillary network. However, unlike the lens, the derivatives of the otic vesicle develop a specific blood supply. The outer wall of the cochlear duct is in direct contact with vascular connective tissue, the stria vascularis (Bast and Anson, '49). The stria vascularis forms as a specialised, stratified epithelium with a rich plexus of intraepithelial capillaries, and may be involved in control of the ionic composition of the endolymph.

It is likely that the virus gains access to the inner ear through the blood supply of the stria vascularis. Examination of fetuses and neonates after rubella infection showed damage to the epithelium of the cochlear duct (Fig. 5) (Gray, '60; Töndury and Smith '66) and the stria vascularis (Nager, '52; Lindsay et al., '54; Ward et al., '68; Brookhouser and Bordley, '73). Typical later findings included cystic dilation of the stria vascularis, and partial collapse of Reissner's vestibular membrane. Collapse of the cochlear duct might be due to changes in quantity or composition of the endolymph (Brookhouser and Bordley, '73). Ongoing viral infection and/or immunological mechanisms, such as immune complex deposition in small vessels, may lead to further damage.

Sensorineural deafness is also the most common handicap caused by congenital cytomegalovirus infection, and is also associated with cytopathology in Reissner's membrane and the stria vascularis and cytomegalovirus-specific immu-



**Fig. 6.** Brain arteriole showing focal destruction of wall with thickening due to deposition of PAS positive acellular material  $\times 240$ . There is nodular elevation of the intima with projection into the lumen. From an infant that died postnatally from CRS. From Rorke ('73) *Arch Otolaryngol* 98:249–251. Copyright 1967–73 American Medical Association.

no fluorescence in the organ of Corti. Postnatal virus infection with mumps can also cause deafness (Ward et al., '68; Lindsay '73), and is associated with damage to the stria vascularis—which is thought to be susceptible due to its relatively slow circulation and its intraepithelial network (Lindsay, '73). The sparing of vestibular function may be due to the absence of a stria vascularis in the vestibular system (Ward et al., '68).

These studies suggest that rubella sensorineural deafness is caused by direct viral damage of the epithelium of the cochlear duct and/or the stria vascularis causing secondary changes to the endolymph and structure of the cochlear duct. Cessation of the sensitive period for deafness due to rubella infection at about 16 weeks may be due to the improved fetal immune response and the transfer of maternal antibodies that has occurred at this stage.

### Brain damage

Brain damage only occurs after rubella infection in the first 16 weeks of gestation, causing mild to severe mental retardation with spastic diplegia (Cooper et al., '69). Gross structural malformations are rare; instead, there is ischemic damage and variable microcephaly. In a review of the pathology of 89 infants with CRS, 46 brains contained some type of vascular abnormality, and 31 of the 46 showed ischemic brain damage (Rorke, '73). The vascular lesions included focal destruction of the walls of cerebral blood vessels (Fig. 6), defects of the internal elastic lamina with overlying proliferation of fibrous tissue, pericapillary collections of granular material, and endothelial proliferation with narrowing of the lumina. Most frequently affected were the small penetrating vessels and the capillary bed within the

basal ganglia; inflammatory changes in the brain were described as minimal at best (Rorke, '73). Necrotic brain tissue, presumably due to ischemia, was usually located in the areas adjacent to the damaged vessels or in their terminal field of supply. Linear hyperechogenic basal ganglia lesions in patients with CRS are a common, but nonspecific, finding—but are suggestive of an earlier vasculopathy (Chang et al., '95).

It is likely that the vascular lesions originate early in development since endothelial damage and small hemorrhages were seen in the embryonic brains of first trimester abortuses (Töndury and Smith, '66). Ongoing viral infection of endothelial cells and/or subsequent damage by immune complexes may lead to progressive vascular damage in the prenatal and perhaps postnatal brain (Chang et al., '95). In vitro studies suggest that human astrocytes are selectively and heavily infected, but such specificity has not been described in vivo (Chantler et al., '95).

A proportion of CRS infants have microcephaly: just under 25% in the series reviewed by Rorke ('73). An MRI study of CRS patients with schizophrenia-like symptoms showed reduced cortical gray matter volume but not white matter volume, and significant ventricular enlargement (Lim et al., '95). The authors argued that if the brain size was simply associated with small somatic size, there would be small gray matter and small ventricular volumes, both commensurate with the small somatic size. In these patients, however, the possibility that the brain pathology was primarily related to the schizophrenia, rather than rubella, was not excluded. Microcephaly in CRS infants would be consistent with the concept that the virus can infect the neuroepithelium and reduce cell proliferation (Naeye and Blanc, '65). Töndury and Smith ('66) also reported reduced cortical width in 10 affected fetuses.

Persistence of the virus in the cerebrospinal fluid may be associated with meningoencephalitis and cerebral vasculitis postnatally (Desmond et al., '66). The encephalitis can persist for years, causing cumulative damage. A delayed manifestation of chronic infection in the brain, beginning 10 or more years after primary infection, is progressive rubella panencephalitis. A neuropathological study of this condition showed vasculitis with fibrinoid necrosis, severe neuronal loss and demyelination, and some old microinfarcts (Townsend et al., '82). Due to the absence of virus or viral antigens in the brain tissue, the authors speculated that deposits of rubella specific IgG within blood vessels may play an important role in the development of vasculitis seen in the brain (Townsend et al., '82). A similar mechanism has been proposed to explain the vasculitis seen in lung and skin of infants with the late-onset rubella syndrome (Tardieu et al., '80) or retinal vasculitis also caused by rubella in a 13-year-old (Riikonen, '95). The involvement of a similar mechanism prenatally is unlikely due to the reported lack of inflammatory reaction seen in the brains of CRS infants (Rorke, '73).

**Fetal growth inhibition**

A constant feature of CRS is fetal growth retardation, perhaps due to reduced or slower cell division in infected cells. In one study birth weights were 65% of control values (Naeye and Blanc, '65) and cell counts of organs from CRS autopsy material revealed that the number of parenchymal cells in the heart, liver, pancreas, and adrenals were reduced by 30–80%.

A number of in vitro studies have shown that the rubella virus is not particularly cytopathic, but the infected cells grow and divide more slowly than do uninfected cells (Frey, '94). Curiously, cell strains derived from different fetal human organs show widely different responses. The most severe effect is seen after rubella infection of diploid cell strains derived from human fetal lungs, kidney, or pituitary which results in cessation of growth within a few passages; while the effects on cells derived from the skin or muscle were inapparent, although these cells had a higher percentage of chromosome breakage (Plotkin et al., '65; Hoskins and Plotkin, '67; Boué and Boué, '69). In vitro growth inhibition has been attributed to a protein (not interferon) present in the culture fluid which inhibited mitosis (Plotkin and Vaheri, '67). More recently an effect of the virus on microfilaments has been proposed as the cause of mitotic inhibition, since in vitro infected cells show disruption of actin filaments (Bowden et al., '87). Other suggested causes of growth inhibition are rubella-induced chromosomal breaks (Chang et al., '66) and changes in responsiveness to growth factors (Yoneda et al., '86).

If most cells in the fetus were infected by the rubella virus, slowing of cell division would result in significant fetal growth retardation. However, it has been shown that only 1 in 10<sup>3</sup> to 10<sup>5</sup> of cells from infected fetal organs harbour the virus (Rawls et al., '68). This concept of clones of infected cells was supported by an immunofluorescence study that examined the distribution of the virus in tissues from a CRS infant who died 6 days after birth (Woods et al., '66). Foci of fluorescence were scattered through the heart and were compact with discrete borders. The infected areas showed no morphologic abnormalities. One focus was found in 40 sections of skeletal muscle, and no fluorescence was detected in brain sections. In general these results are consistent with the focal nature of much of the pathology seen in infected fetuses. Other explanations for fetal growth retardation include placental impairment and vascular insufficiency.

**SUMMARY**

It is apparent that there are many unanswered questions about the pathogenesis of CRS. For instance, the chance of embryonic infection decreases in the second semester only to increase again in the third trimester. This is presumably due to unspecified changes in the placenta. When the embryo is infected early in the first trimester it does not appear to have any conventional immunological response to prevent spread of the virus. Yet it has been suggested that only 1 in 10<sup>3</sup> to 10<sup>5</sup> of its cells become infected. If this is true, what controls the spread of the virus

in the early embryo? Why does the virus not affect major morphogenetic processes?

There is considerable evidence that the virus spreads through the vascular system of the infected fetus and the observed cardiovascular, CNS, and hearing defects may be primarily due to focal cytopathic damage to the walls of blood vessels and lining of the heart; subsequent organ infection and/or ischemia may lead to further damage. Damage to blood vessels is probably extensive throughout the fetus and may be the cause of the generalized growth retardation. The effects in the eye appear to be due to a direct cytopathic effect, particularly on the lens. The short susceptible period for cataract formation is consistent with the protective effect of the lens capsule.

Deafness, cardiovascular and neurological damage, and retinopathy all occur when infection takes place in the first 16 weeks of gestation and are rare after this time, despite the absence of any obvious morphological or functional changes in the susceptible structures. This termination of susceptibility in the second trimester is consistent with development of the fetal immune response and increased transfer of maternal IgG. The effect on blood vessels in particular may be limited by antibody production, although existing endothelial infection and damage may be progressive. The fetus seems unable to rid itself of established intracellular virus.

The causes of the well-established late manifestations remain unknown. If these serious late-appearing effects are due to prenatal damage, then it is possible that other human teratogens may also cause unexpected late symptoms. This should also be a concern in the area of animal reproductive toxicology testing.

**ACKNOWLEDGMENTS**

The author gratefully acknowledges the advice and help given by Dr. M. Burgess in the preparation of this manuscript.

**LITERATURE CITED**

Alford, C.A., F.A. Neva, and T.H. Weller (1964) Virologic and serologic studies on human products of conception after maternal rubella. *N. Eng. J. Med.*, 271:1275–1281.

Alford, C.A., J. Schaffer, W.J. Blankenship, J.V. Straumfjord, and G. Cassady (1967) A correlative immunologic, microbiologic and clinical approach to the diagnosis of acute and chronic infections in newborn infants. *N. Eng. J. Med.*, 277:437–449.

Amstey, M.S. (1969) Intra-amniotic inoculation of rubella virus. *Am. J. Obstet. Gynecol.*, 104:573–577.

Avila, L., W.E. Rawls, and P.B. Dent (1972) Experimental infection with rubella virus. I. Acquired and congenital infection in rats. *J. Infect. Dis.*, 126:585–592.

Barber, A.N. (1955) *Embryology of the Human Eye*. St. Louis: C.V. Mosby Co.

Bast, T.H., and B.J. Anson (1949) *The Temporal Bone and The Ear*. Springfield, IL. Charles C. Thomas.

Bellantì, J.A., M.S. Artenstein, L.C. Olson, E.L. Buescher, C.E. Luhrs, and K.L. Milstead (1965) Congenital rubella: Clinicopathologic, virologic and immunologic studies. *Am. J. Dis. Child.*, 110:464–472.

Best, J.M., and J.E. Banatvala (1995) Rubella. In: *Principles and Practice of Clinical Virology*, 3rd Ed. A.J. Zuckerman, J.E. Banatvala, and J.R. Pattison, eds. John Wiley and Sons Ltd. Chichester, U.K., pp. 363–400.

- Boniuk, M., and L.E. Zimmerman (1967) Ocular pathology in the rubella syndrome. *Arch. Ophthalmol.*, 77:455-473.
- Bouchard, C.J., and A. Charrin (1886) La cataracte produite par la naphthaline. *C.R. Soc. Biol.*, 8:614-615.
- Boué, A., and J.G. Boué (1969) Effects of rubella virus infection on the division of human cells. *Am. J. Dis. Child.*, 118:45-48.
- Bowden, D.S., J.S. Pedersen, B.H. Toh, and E.G. Westaway (1987) Distribution by immunofluorescence of viral products and actin-containing cytoskeletal filaments in rubella virus infected cells. *Arch. Virol.*, 92:211-219.
- Braun, C., D. Kampa, R. Fressle, E. Willke, M. Stahl, and O. Haller (1994) Congenital rubella syndrome despite repeated vaccination of the mother: A coincidence of vaccine failure with failure to vaccinate. *Acta Paediatr.*, 83:674-677.
- Brookhouser, P.E., and J.E. Bordley (1973) Congenital rubella deafness. *Arch. Otolaryngol.*, 98:252-257.
- Burgess, M.A. (1990) Rubella vaccination just before or during pregnancy. *Med. J. Australia*, 152:507-508.
- Burgess, M.A. (1991) Gregg's rubella legacy 1941-1991. *Med. J. Australia*, 155:355-357.
- Catalano, L.W., D.A. Fuccillo, R.G. Traub, and J.L. Sever (1971) Isolation of rubella virus from placentas and throat cultures of infants. A prospective study after the 1964-65 epidemic. *Obstet. Gynecol.*, 38:6-14.
- Centers for Disease Control, U.S. Department of Health and Human Services. (1989) Rubella vaccination during pregnancy—United States, 1971-1988 *MMWR* 38:289-293.
- Chang, T.H., P.S. Moorehead, J.G. Boué, S.A. Plotkin, and J.M. Hoskins (1966) Chromosome studies of human cells infected in utero and in vitro with rubella virus. *P. Soc. Exp. Biol. Med.*, 122:236-243.
- Chang, Y.-C., C.-C. Huang, and C.-C. Liu (1995) Frequency of linear hyperchogenicity over the basal ganglia in young infants with congenital rubella syndrome. *Clin. Inf. Dis.*, 22:569-571.
- Chantler, J.K., L. Smyrnis, and G. Tai (1995) Selective infection of astrocytes in human glial cell cultures by rubella virus. *Lab. Invest.*, 72:334-340.
- Cooper, L.Z. (1968) Rubella: A preventable cause of birth defects. In: *Birth Defects Original Article Series*. D. Bergsma, eds. Vol. IV, No. 7, pp. 23-25. National Foundation, New York.
- Cooper, L.Z., P.R. Ziring, A.B. Ockerse, B.A. Fedun, B. Kiely, and S. Krugman (1969) Rubella, clinical manifestations and management. *Am. J. Dis. Child.*, 118:18-29.
- Cordes, F.C., and A. Barber (1966) Changes in the lens of an embryo after rubella. *Arch. Ophthalmol.*, 36:135-140.
- Cotlier, E., J. Fox, G. Bohigian, C. Beaty, and A. du Pree A (1968) Pathogenic effects of rubella virus on embryos and newborn rats. *Nature*, 217:38-40.
- Delahunt, C.S., and N. Rieser (1967) Rubella-induced embryopathies in monkeys. *Am. J. Obstet. Gynecol.*, 99:580-588.
- Desmond, M.M., G.S. Wilson, J.L. Melnick, D.B. Singer, T.E. Zion, A.J. Rudolph, R.G. Pineda, M.-H. Ziai, and R.J. Blattner (1967) Congenital rubella encephalitis. *Pediatrics*, 71:311-331.
- Driscoll, S.G. (1969) Histopathology of gestational rubella. *Amer. J. Dis. Child.*, 118:49-53.
- Dudgeon, J.A. (1969) Congenital rubella: Pathogenesis and immunology. *Am. J. Dis. Child.*, 118:35-44.
- Durandy, A., C. Oury, C. Griscelli, Y. Dumez, J.F. Oury, and R. Henrion (1982) Prenatal testing for inherited immune deficiencies by fetal blood sampling. *Prenatal Diag.*, 2:109-113.
- Duke-Elder, W.S. (1938) *Text-Book of Ophthalmology*. Vol II. Clinical Methods of Examination, Congenital and Developmental Anomalies, General Pathological and Therapeutic Considerations, Diseases of the Outer Eye. Henry Kimpton, London, p. 1366.
- Editorial (1941) Congenital cataract following German measles in the mother. *Med. J. Australia*, 2:651-652.
- Editorial (1944) Rubella and congenital malformations [annotation] *Lancet*, 1:316.
- Editorial (1945) Congenital defects and rubella. *Brit. Med. J.*, 1:635-636.
- Editorial (1946) Congenital defects following maternal rubella. *J.A.M.A.*, 130:574-575.
- Enders, G. (1985) Serologic test combinations for safe detection of rubella infections. *Rev. Infect. Dis.*, 7:S113-S122.
- Enders, G., U. Nickler-Pacher, E. Miller, and J.E. Cardock-Watson (1988) Outcome of confirmed periconceptual maternal rubella. *Lancet*, 1:1445-1447.
- Esterly, J.R., and E.H. Oppenheimer (1967) Vascular lesions in infants with congenital rubella. *Circulation*, 36:544-554.
- Esterly, J.R., and E.H. Oppenheimer (1973) The pathologic manifestations of intrauterine rubella infection. *Arch. Otolaryngol.*, 98:246-248.
- Fabiyi, A., G.L. Gitnick, J.L. Sever (1967) Chronic rubella virus infection in the ferret (*Mustela putorius furo*) puppy. *P. Soc. Exp. Biol. Med.*, 125:766-771.
- Forrest, J.M., M.A. Menser, and J.D. Harley (1969a) Diabetes mellitus and congenital rubella. *Pediatrics*, 44:445-447.
- Forrest, J.M., M.A. Menser, and R.D.K. Reye (1969b) Obstructive arterial lesions in rubella. *Lancet*, 1:1263-1265.
- Forrest, J.M., M.A. Menser, and J.A. Burgess (1971) High frequency of diabetes mellitus in young adults with congenital rubella. *Lancet*, 1:332-334.
- Frey, T.K. (1994) Molecular biology of rubella virus. *Adv. Virus Res.*, 44:69-160.
- Garcia, A.G.P., R.L.S. Marques, Y.Y. Lobato, E.F. Fonesca, and M.D. Wigg (1985) Placental pathology in congenital rubella. *Placenta*, 6:281-295.
- Gilbert, G.L. (1991) Rubella. In: *Infectious Disease in Pregnancy and the Newborn Infant*, Harwood Academic Publishers, Chur, Switzerland, pp. 23-62.
- Gitlin, D., and A. Biasucci (1969) Development of  $\gamma$ G,  $\gamma$ A,  $\gamma$ M,  $\beta_{1c}/\beta_{1A}$ , C'1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen,  $\alpha$ 1-antitrypsin, orosomucoid,  $\beta$ -lipoprotein,  $\alpha$ 2-macroglobulin and prealbumin in the human conceptus. *J. Clin. Invest.*, 48:1433-1446.
- Givens, K.T., D.A. Lee, T. Jones, and D.M. Ilstrup (1993) Congenital rubella syndrome: Ophthalmic manifestations and associated systemic disorders. *Br. J. Ophthalmol.*, 77:358-363.
- Gray, J.E. (1960) Rubella in pregnancy. A report on six embryos. *Brit. Med. J.*, 1:1388-1392.
- Gregg, N.Mc.A. (1941) Congenital cataract following German measles in the mother. *Trans. Ophthalmol. Soc. Australia*, 3:35-46.
- Hanshaw, J.B., J.A. Dudgeon, and W.C. Marshall (1985) Rubella. In: *Viral Diseases of the Fetus and Newborn*, 2nd Edition. W.B. Saunders Co., Philadelphia, pp. 32-69.
- Hoskins, J.M., and S.A. Plotkin (1967) Behavior of rubella virus in human diploid cell strains. II. Studies of infected cells. *Arch. fur die Gesamte Virusforschung*, 21:296-308.
- Howson, C., M. Katz, R. Johnston, and H. Fineberg (1992) Chronic arthritis after rubella vaccination. *Clin. Infect. Dis.*, 15:307-312.
- Jauniaux, E., D. Jurkovic, B. Gulbis, C. Liesnard, C. Lees, and S. Campbell (1995) Materno-fetal immunoglobulin transfer and passive immunity during the first trimester of human pregnancy. *Human Reprod.*, 10:3297-3300.
- Kaplan, C. (1993) The placenta and viral infections. *Sem. Diag. Pathol.*, 10:232-250.
- Karkinen-Jääskeläinen, M., L. Saxén, A. Vaheri, and P. Leinikki (1975) Rubella cataract in vitro: Sensitive period of the developing human lens. *J. Exp. Med.*, 141:1238-1248.
- Kono, R., M. Hibi, Y. Hayakawa, and K. Ishii (1969) Experimental vertical transmission of rubella virus in rabbits. *Lancet*, 1:343-347.
- Kresky, B., and J.S. Nauheim (1967) Rubella retinitis. *Am. J. Dis. Child.*, 113:305-310.
- Lancaster, P.A.L. (1992) The eyes have it: Norman McAlister Gregg and congenital rubella. In: *Rubella—Essays in Honour of the Centenary of the Birth of Sir Norman McAlister Gregg 1892-1966*, The Royal Australian College of Physicians, Sydney, pp. 25-50.
- Lebon, P., F. Daffos, A. Checoury, L. Grangeot-Keros, F. Forestier, and J.E. Toubanc (1985) Presence of an acid-labile alpha-interferon in sera from fetuses and children with congenital rubella. *J. Clin. Microbiol.*, 21:775-778.
- Lim, K.O., D.M. Beal, R.L. Harvey, T. Myers, B. Lane, E.V. Sullivan, W.O. Faustman, and A. Pfefferbaum (1995) *Biol. Psychiatry*, 37:764-776.
- Lindsay, J.R., D.G. Caruthers, W.G. Hemenway, and S. Harrison (1954) Inner ear pathology following maternal rubella. *Ann. Otol. Rhinol. Laryngol.*, 62:1201-1218.
- Lindsay, J.R. (1973) Histopathology of deafness due to postnatal viral disease. *Arch. Otolaryngol.*, 98:258-264.
- Lobach, D.F., L.L. Hensley, W. Ho, and B.F. Haynes (1985) Human T cell antigen expression during the early stages of fetal thymic maturation. *J. Immunol.*, 135:1752-1759.

- London, W.T., D.A. Fuccillo, B. Anderson, and J.L. Sever (1970) Concentration of rubella virus antigen in chondrocytes of congenitally infected rabbits. *Nature*, 226:172-173.
- Lundström, R. (1962) Rubella during pregnancy: A follow-up study of children born after an epidemic of rubella in Sweden, 1951, with additional investigations on prophylaxis and treatment of maternal rubella. *Acta Paediatr.* 51(suppl 133):1-110.
- Machado, R., A. Aguilera, E. Cutie, P. Hoyo, and G. Kouri (1976) Morphologic alterations of experimental rubella syndrome in rabbits. *Morphol. Embryol.*, 22:41-45.
- Mann, I. (1937) *Developmental Abnormalities of the Eye*. London: Cambridge University Press, p. 18.
- McIntosh, E.D., and M.A. Menser (1992) A fifty-year follow-up of congenital rubella. *Lancet*, 340:414-415.
- Menser, M.A., L. Dods, and J.D. Harley (1967a) A twenty-five year follow-up of congenital rubella. *Lancet*, 2:1347-1350.
- Menser, M.A., J.D. Harley, R. Hertzberg, D.C. Dorman, and A.M. Murphy (1967b) Persistence of virus in lens for three years after prenatal rubella. *Lancet*, 2:387-388.
- Menser, M.A., D.C. Dorman, K.G. Kenrick, S.G. Purvis-Smith, R.F. Slinn, L. Dods, and J.D. Harley (1969) Congenital rubella. Long term follow-up study. *Am. J. Dis. Child.*, 118:32-34.
- Menser, M.A., and R.D. K. Reye (1974) The pathology of congenital rubella: A review written by request. *Pathol.*, 6:215-222.
- Menser, M.A., J.M. Forrest, and R.D. Bransby (1978) Rubella infection and diabetes mellitus. *Lancet*, 1:57-60.
- Miller, E., J.E. Cradock-Watson, and T.M. Pollock (1982) Consequences of confirmed maternal rubella at successive stages of pregnancy. *Lancet*, 2:781-784.
- Naeye, R.L., and W. Blanc (1965) Pathogenesis of congenital rubella. *J.A.M.A.*, 194:1277-1283.
- Nager, F.R. (1952) Histologic studies of the ears of children born after rubella in pregnancy. *Pract. Otorhinolaryngol.*, 14:337-359.
- Notarangelo, L.D., G. Chirico, A. Chiara, A. Colombo, G. Rondini, A. Plebani, A. Martini, and A.G. Ugazio (1984) Activity of classical and alternative pathways of complement in preterm and small for gestational age infants. *Pediatr. Res.*, 18:281-285.
- O'Rahilly, R. (1983) The timing and sequence of events in the development of the human eye and ear. *Anat. Embryol.*, 168:87-99.
- Ornoy, A., S. Segal, M. Nishmi, A. Simcha, and W.Z. Polishuk (1973) Fetal and placental pathology in gestational rubella. *Am. J. Obstet. Gynecol.*, 116:946-956.
- Parkman, P.D., P.E. Phillips, and H.M. Meyer (1965) Experimental rubella virus infection in pregnant monkeys. *Am. J. Dis. Child.*, 110:390-394.
- Patterson, R.L., A. Koren, and R.L. Northrop (1973) Experimental rubella virus infection of marmosets (*saguinus species*). *Lab. Anim. Sci.*, 23:68-71.
- Plotkin, S.A., A. Boué, and J.G. Boué (1965) The in vitro growth of rubella virus in human embryo cells. *Am. J. Epidemiol.*, 81:71-85.
- Plotkin, S.A., and A. Vaheri (1967) Human fibroblasts infected with rubella virus produce a growth inhibitor. *Science*, 156:659-661.
- Plotkin, S.A. (1975) Routes of fetal infection and mechanisms of fetal damage. *Am. J. Dis. Child.*, 129:444-449.
- Preblud, S.R., and C.A. Alford (1990) Rubella. In: *Infectious Diseases of the Fetus and Newborn Infant*, 3rd Ed. J.S. Remington and J.O. Klein eds. W.B. Saunders Co., Philadelphia, pp. 196-240.
- Rawls, W.E., J. Desmyter, and J.L. Melnick (1968) Virus carrier cells and virus free cells in fetal rubella. *P. Soc. Exp. Biol. Med.*, 129:477-483.
- Rawls, W.E. (1974) Viral persistence in congenital rubella. *Prog. Med. Virol.*, 18:273-288.
- Rayfield, E.J., K.J. Kelly, and J.-W. Yoon (1986) Rubella virus-induced diabetes in the hamster. *Diabetes*, 35:1278-1281.
- Riikonen, R.S. (1995) Retinal vasculitis caused by rubella. *Neuropediatr.*, 26:174-176.
- Rorke, L.B., A. Fabiyi, T.S. Elizan, and J.L. Sever (1968) Experimental cerebrovascular lesions in congenital and neonatal rubella-virus infections of ferrets. *Lancet*, 2:153-154.
- Rorke, L.B. (1973) Nervous system lesions in the congenital rubella syndrome. *Arch Otolaryngol.*, 98:249-251.
- Royo, C., J.H. Touraine, and O. de Bouteiller (1987) Ontogeny of T cell lymphocyte differentiation in the human fetus. Acquisition of phenotype and functions. *Thymus*, 10:57-73.
- Schardein, J.L. (1993) *Chemically induced birth defects*, 2nd edition. Marcel Dekker Inc., New York, pp. 228-229.
- Sever, J.L., G.W. Meier, W.F. Windle, G.M. Schiff, G.R.G. Monif, and A. Fabiyi (1966) Experimental rubella in pregnant rhesus monkeys. *J. Infect. Dis.*, 116:21-26.
- Sever, J.L., M.A. South, and K.A. Shaver (1985) Delayed manifestations of congenital rubella. *Rev. Infect. Dis.*, 7:S164-S169.
- Singer, D.B., A.J. Rudolph, H.S. Rosenberg, W.E. Rawls, and M. Boniuk (1967) Pathology of the congenital rubella syndrome. *Pediatrics*, 71:665-675.
- South, M.A., and J.L. Sever (1985) Teratogen update: The congenital rubella syndrome. *Teratology*, 31:297-307.
- Swan, C., A.L. Tostevin, B. Moore, H. Mayo, and G.H.B. Black (1943) Congenital defects in infants following infectious diseases during pregnancy. With special reference to the relationship between German measles and cataract, deaf mutism, heart disease and microcephaly, and to the period of pregnancy in which the occurrence of rubella is followed by congenital abnormalities. *Med. J. Australia*, 2:201-210.
- Swan, C. (1944) A study of three infants dying from congenital defects following maternal rubella in the early stages of pregnancy. *J. Pathol. Bacteriol.*, 61:289-295.
- Tardieu, M., B. Grosperre, A. Durandy, and C. Griseilli (1980) Circulating immune complexes containing rubella antigens in late-onset rubella syndrome. *J. Pediatr.*, 97:370-373.
- Töndury, G., and D.W. Smith (1966) Fetal rubella pathology. *J. Pediatr.*, 68:867-879.
- Townsend, J.J., W.G. Stroop, J.R. Baringer, J.S. Wolinsky, J.H. McKerrow, and B.O. Berg (1982) Neuropathology of progressive rubella panencephalitis after childhood rubella. *Neurology*, 32:185-190.
- Ueda, K., Y. Nishida, and K. Oshinia (1979) Congenital rubella syndrome: Correlation of gestational age at time of maternal rubella with type of defect. *J. Pediatr.*, 94:763-765.
- Ueno, Y., T. Miyawaki, H. Seki, A. Matsuda, K. Taga, H. Sato, and N. Taniguchi (1985) Differential effects of recombinant human interferon- $\gamma$  and interleukin 2 on natural killer cell activity of peripheral blood in early human development. *J. Immunol.*, 135:180-184.
- Van der Hoeve, J. (1913) Wirkung von naphthol auf die und auf foetale augen. *Graef. Arch. Ophthalm.*, 85:305-315.
- Ward, P.H., V. Honrubia, B.S. Moore (1968) Inner ear pathology in deafness due to maternal rubella. *Arch. Otolaryngol.*, 87:22-28.
- Warkany, J. (1973) Trends in teratological research. In: *Pathobiology of Development or Ontogeny* Revisted. V.D. Perrin and M.J. Finegold, eds. William & Wilkins Co., Baltimore, pp. 1-10.
- Wesselhoft, C. (1947a) Rubella (German measles). *N. Eng. J. Med.*, 236:943-950.
- Wesselhoft, C. (1947b) Rubella (German measles). *N. Eng. J. Med.*, 236:978-988.
- Wilson, C.B. (1990) Developmental immunobiology and the role of host defenses in neonatal susceptibility. In: *Infectious Diseases of the Fetus and newborn infants*, 3rd ed. J.S. Remington and J.O. Klein, eds. W.B. Saunders Co., Philadelphia, pp. 17-67.
- Winkelstein, J.A. (1992) The complement system in the fetus and newborn. In: *Fetal and Neonatal Physiology*, Volume 2. R.A. Polin and W.W. Fox, eds. W.B. Saunders Co., Philadelphia, pp. 1470-1475.
- Wolinsky, J.S. (1990) Rubella. In: *Virology*, 2nd edition. B.N. Fields and D.M. Knipe, eds. Raven Press Ltd., New York, pp. 815-838.
- Woods, W.A., R.T. Johnson, D.D. Hostetler, M.L. Lepow, and F.C. Robbins (1966) Immunofluorescent studies on rubella infected tissue cultures and human tissues. *J. Immunol.*, 96:253-260.
- Yarchoan, R., and D.L. Nelson (1983) A study of the functional capabilities of human neonatal lymphocytes in vitro specific antibody production. *J. Immunol.*, 131:1222-1228.
- Yoneda, T., M. Urade, M. Sakuda, and T. Miyazaki (1986) Altered growth, differentiation, and responsiveness to epidermal growth factor of human embryonic mesenchymal cells of palate by persistent rubella virus infection. *J. Clin. Invest.*, 77:1613-1621.
- Zimmerman, L.E. (1965) Pathogenesis of rubella cataract. *Arch. Ophthalmol.*, 73:761-763.