RUBELLA IN PREGNANCY

VIRUS ISOLATION AND INCLUSION BODIES

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Although the causation of many foetal abnormalities still remains a mystery, some abnormalities are clearly due to genetic factors, in others environmental conditions play a part, and in a few infection of foetus is the basis of the abnormality. The teratogenetic mechanism of rubella has now been established by the isolation of the rubella virus from a human embryo.[1] The spread of infection is probably from the decidual tissues.

During the recent, continuous, widespread epidemic of rubella over a period of 10 months in Cape Town, the rubella virus was isolated from the garglings of several patients and the opportunity also arose to examine the placental tissues of a few of the many women who developed the infection during the first trimester of pregnancy. The rubella virus was isolated from one embryo and the placental tissues of 4 women, and inclusion bodies were demonstrated in decidual cells.

MATERIAL AND METHODS

The material consisted of chorionic villi and sheets of decidual tissue. These were obtained from women who had recently had rubella in the early months of pregnancy. In addition there was a foetus aged 8 weeks in the case of Wal and one aged 12 weeks in the case of Hau. The latter had been dead *in utero* for apparently 24 hours before expulsion. Table I indicates the duration of pregnancy in these women and the stage at which rubella manifested itself.

TABLE I. DURATION OF PREGNANCY AND STAGE AT WHICH RUBELLA MANIFESTED ITSELF

Name	*Pregnancy (duration)*	*Rubella before termination*	*Virus*	
Wal	8 weeks	10 days	+	
Fin	7 „	10 „	+	
Has	6-7 „	14 „	+	
Kni	12 „	10 „	+	
Hat	12 „	63 „	—	
Hau	12 „	14 „	—	(cataracts)

+ = virus isolated, — = no virus.

A portion of the material was fixed in Bouin's fluid for histological examination. The rest was incubated at room temperature for 60 minutes with penicillin, 1,000 u./ml., and streptomycin, 1 mg./ml. The material was then stored at −70°C. until tested.

The placental material was emulsified in a TenBroeck grinder in Hanks' balanced salt solution plus 1% bovine plasma albumin to form approximately a 20% emulsion. This was spun at 1,500 r.p.m. for 15 minutes and the supernatant fluid used for inoculation. The general principles described by Buescher and Parkman[2] for the isolation of rubella virus were employed in this investigation.

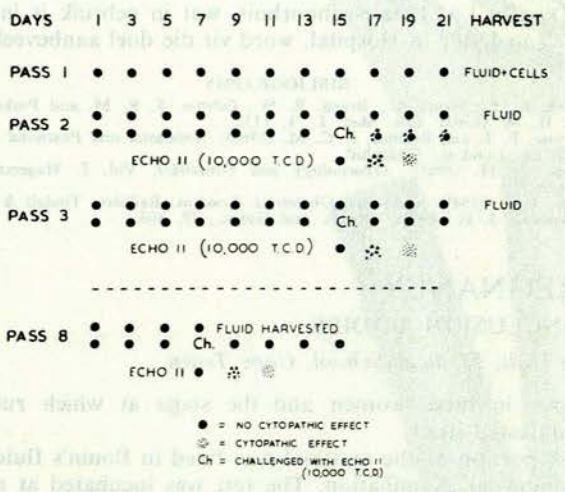
Tissue culture. Only primary digests of kidney cells from the Vervet monkey (*Cercopithicus aethiops pygerythrus*) were used (MK). These were prepared as monolayer cultures in flat 2-oz. bottles.

Two ml. of the prepared placental tissues were inoculated into each tissue culture bottle which was left at room temperature for an hour to allow adsorption of virus to cells. An equal volume of nutrient medium (consisting of Hanks' balanced salt solution with 0·5% lactalbumin hydrolysate, 4% chicken-serum and 0·25% bovine plasma albumin) was then added. The cell cultures were incubated at 35°C. for 14 - 21 days to allow virus growth. The fluid was changed every 4 - 5 days.

The first passage material consisted of fluid and cells and subsequent passages of fluid only. Some cultures were maintained for 3 - 4 weeks, and at no time was any cytopathic effect (degenerative change indicative of virus multiplication) observed. The presence of virus was demonstrated by interference with the growth of Echo 11 virus, which regularly produces cytopathic effects in monkey kidney cells.

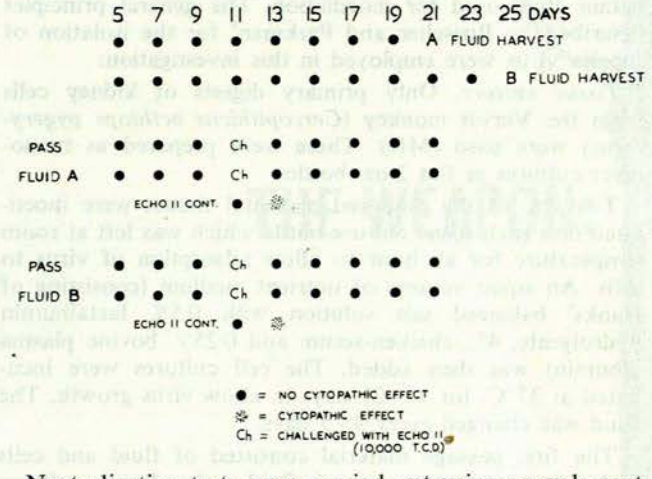
Echo 11 challenge. Challenge with Echo 11 virus was accomplished by inoculating MK cultures infected with rubella and an equal number of control uninfected cultures with 10,000 tissue culture infectious doses ($TCID_{50}$) of Echo 11 virus in 1-ml. volume. Only one-half of the bottles corresponding to any given sample were challenged while the rest were used for further passages. The cultures were maintained for a further 3 days after the control Echo 11 bottles had shown complete cytopathic effect (Table II).

TABLE II. (SEE TEXT)



To assess the *duration of virus excretion* from tissues, the fluid removed from the MK cultures on the 21st and 25th days of incubation from case Wal were passaged, and after 10 days these new cultures were challenged with Echo 11 virus (Table III).

TABLE III. (SEE TEXT)



Neutralization tests were carried out using convalescent sera from patients with rubella infection.

RESULTS AND COMMENT

Histology

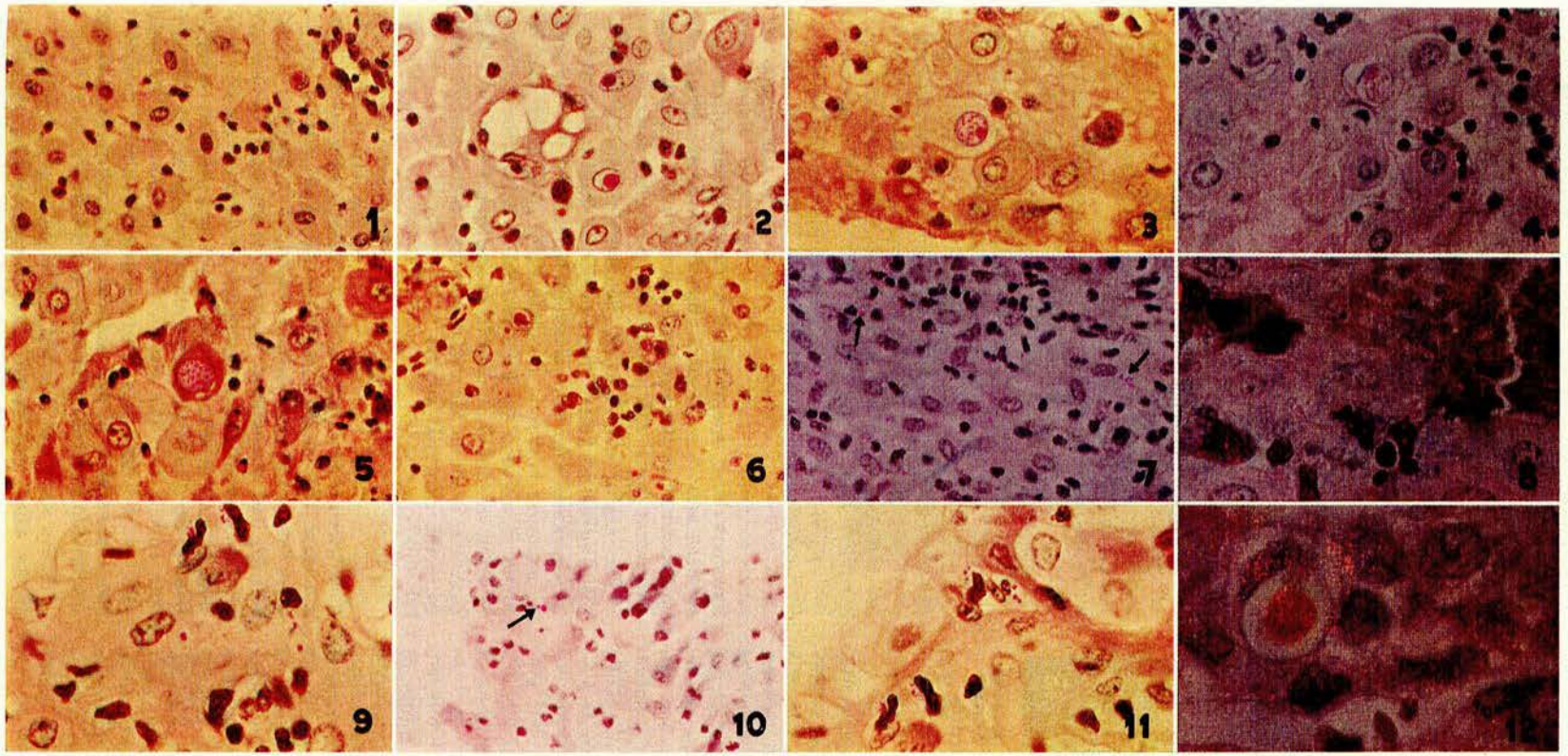
The placental tissues, consisting of chorionic villi and sheets of decidual cells, were in a good state of preservation. They were fixed in Bouin's fluid and sections were stained with haematoxylin and eosin. There was no necrosis and only a negligible inflammatory cell infiltration consisting of lymphocytes. The chief feature was the presence of inclusion bodies. These were demonstrable in decidual cells only and not in chorionic villi nor in endometrial glands. The number of cells containing inclusion bodies varied from moderate collections scattered over several fields to single cells which were found only after a prolonged search. Cytoplasmic inclusions were found in all cases except Hat. The placental tissues of Hau were not received. In one case (Kni) both intranuclear and cytoplasmic inclusions were found. Some cells showed vesiculation or so-called ballooning degeneration of nuclei which, in itself, suggests a virus infection.

The cytoplasmic inclusion bodies were acidophilic, sharply defined, and generally very small. They were seldom larger than 2μ in diameter and surrounded by a clear halo (Figs. 9, 10, 12). They varied in number from 1 or 2 to about half-a-dozen or more. The majority of cytoplasmic inclusion bodies were associated with a dark nucleus resembling that of a stromal cell (Figs. 8, 11). In some the appearance suggested a pyknotic nucleus of a decidual cell, while others were clearly in the cytoplasm of normal-looking decidual cells (Figs. 7, 9, 12). They differed in position from the occasional acidophilic granules found in normal stromal endometrial tissue in that they were haphazard in distribution (Fig. 14) and not paranuclear.

In the case of Kni, there was a round, brightly acidophilic intranuclear body resembling a conspicuous nucleolus when small (Fig. 1). These inclusion bodies were either at the centre or towards the periphery of the nuclei; they were round, oval or occasionally irregular in shape, and the outline was frequently ill-defined (Fig. 4). They varied in size from a small dot to a large body occupying the major portion of the nucleus. There was margination of the basichromatin on the nuclear membrane which was often separated from the inclusion body by clear nucleoplasm (Figs. 2, 6). In an occasional cell, the inclusion body occupied the whole nucleus so that only a thin dark rim of nuclear membrane was recognizable. Careful differential staining showed that this central body was not always homogeneous, but composed of innumerable, minute, rounded particles which were closely aggregated into a morula-like mass (Figs. 3, 5). Exceptionally, both intranuclear and cytoplasmic inclusion bodies occurred in the same cell (Fig. 6).

Examination of products of gestation from 50 consecutive cases received in 1961 in our routine diagnostic work failed to reveal similar inclusion bodies.

The eyes of foetus Hau were fixed in Bouin's and also stained with haematoxylin and eosin. These showed the development of cataract in both eyes, and near the periphery of the lens were small, deeply acidophilic bodies surrounded by a clear halo (Fig. 13). These hyaline bodies are not seen in the usual cataract nor have they been found in the eyes of several normal foetuses of the same age. Similar bodies have been described by Tondury in the lens of foetuses from cases of rubella.[3]



Figs. 1-12. Sections of decidual tissue showing eosinophilic inclusions.

Fig. 1. Small intranuclear inclusion in cell in upper right field (× 340).

Fig. 2. Two oval-shaped intranuclear inclusions (× 340).

Fig. 3. Granular intranuclear inclusions varying in size (× 340).

Fig. 4. Irregular-shaped intranuclear inclusion in cell just above centre (× 340).

Fig. 5. Granular intranuclear inclusion in centre of field (× 340).

Fig. 6. Decidual cell showing both intranuclear and cytoplasmic inclusions (× 340).

Fig. 7. The arrows point to some of the scattered small cytoplasmic inclusions (× 270).

Fig. 8. 2 cytoplasmic inclusions in stromal-like cell just below centre (× 340).

Fig. 9. Small solitary cytoplasmic inclusions in decidual cell in the centre of field and on the right (× 340).

Fig. 10. The arrow points to 2 cytoplasmic inclusions (× 212)

Fig. 11. Small cytoplasmic inclusions above centre (× 340).

Fig. 12. Large cytoplasmic inclusion in decidual cell (× 686).

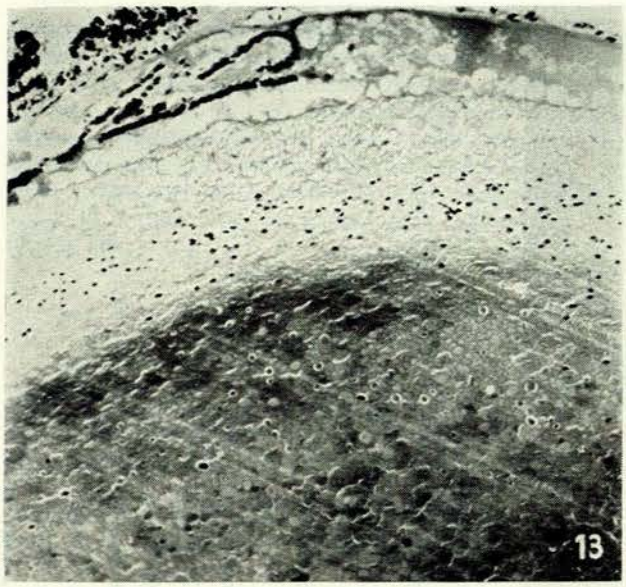


Fig. 13. Cataract from case Hau. Section of eye showing degeneration of lens in lower half of field and in it many small (deeply eosinophilic) bodies, each surrounded by a clear halo.

Virus Isolation

The presence of virus in placental tissues grown in MK cultures was demonstrated by interference with the growth of Echo 11 virus. This interference phenomenon was only partial in the second, and sometimes the third passage, but thereafter was complete. With repeated passage the incubation period became shorter so that interference with Echo 11 virus could be demonstrated earlier—on the 5th to 7th day of the 8th passage (Table II).

There are many problems associated with the isolation of the rubella virus. It is an unstable virus which rapidly loses its infectivity at room temperature and even at 4°C. Unless the material is received soon the chances of isolating the virus are slender. It is only after several passages in tissue culture (which takes many weeks) that the titre of virus present is sufficiently high to produce a satisfactory neutralization test.

A virus interfering with the growth of Echo 11 was isolated from the embryo of Wal, the placental tissues of Wal, Fin, Has and Kni, but not from Hat nor the foetus of Hau (Table I). Failure to isolate the virus from the 2 last-mentioned cases is not surprising since the rubella infection had occurred 63 days previously in the case of Hat and the foetus of Hau had been dead for at least 24 hours before expulsion from the uterus. In the other patients the rubella infection had occurred within 14 days before the cessation of pregnancy. All were from 6 to 12 weeks pregnant. The interference phenomenon was inhibited by convalescent sera from rubella patients.

A few cases of an abnormal infant being born to women who had rubella 2 - 3 weeks before conception have been recorded.[4,5] There has been a great deal of speculation about the pathogenesis of such lesions. Table III shows that the rubella virus continues to grow and be liberated by MK cells for several weeks. Inclusion bodies

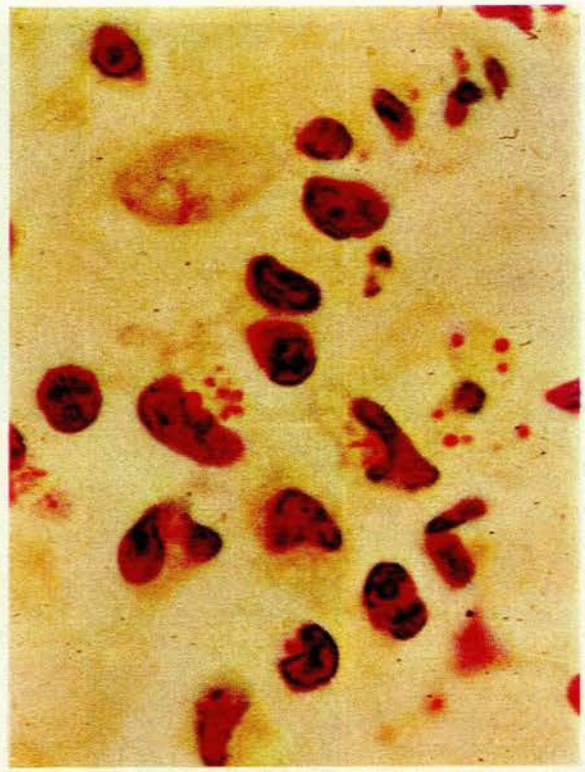


Fig. 14. Section of decidual tissue showing eosinophilic cytoplasmic inclusions (× 1536).

have now been demonstrated in decidual cells of pregnant women, and it is possible that the stromal cells of the endometrium of the non-pregnant uterus harbour the virus and infect the foetus when it develops at a later date.

The above investigation demonstrates virus isolation from an embryo and from placental material, consisting of chorionic villi and decidual tissue, and the presence of inclusion bodies in decidual cells. It is likely that the foetus is invaded by infection from these tissues. Virus invasion of human embryonic tissue appears to be a fundamental event in the pathogenesis of foetal abnormalities from the maternal rubella.

SUMMARY

The rubella virus was isolated from 1 embryo and the placental tissues of 4 women, who had rubella 10 - 14 days before the cessation of pregnancy in the first trimester. All had eosinophilic cytoplasmic inclusion bodies and one showed striking intranuclear inclusion as well. In a fifth case, in whom there were no demonstrable inclusion bodies, the virus was not isolated but here the infection with rubella had occurred 63 days previously. The sixth case was a foetus only. This foetus had 'rubella' cataracts, but the virus was not isolated presumably because the foetus had been dead *in utero* for 24 hours.

Active virus was detected in MK cultures as late as 3 - 4 weeks after inoculation. This long survival of rubella virus could account for reported cases of foetal defects

where the mother had rubella a few weeks *before* conception.

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