Another Case of Mistaken Identity: Rubella and Mycoplasma

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The particles tentatively identified as rubella virus by Chatterji, Beswick & Chapman (1969) were cells of mycoplasma.

When the paper on the morphology of rubella virus by Chatterji et al. (1969) was written, there was no general agreement as to the structure of this virus. The available published accounts indicated a somewhat pleomorphic particle between 500 and 1000 Å in diameter. The particles which these authors tentatively identified as rubella virus fulfilled these criteria, although only a minority showed the ring-like structure with a central nucleoid now known to be characteristic (Murphy, Halonen & Harrison, 1968; Bonissol & Sisman, 1968; Oshiro, Schmidt & Lenette, 1969; Vaheri et al. 1969; von Bonsdorff & Vaheri, 1969).

Chatterji et al. (1969) considered that the particles they observed might have been cells of a mycoplasma but they rejected this because they failed to cultivate any organism from the tissue cultures used (RK 13; Beale, Chrostofinis & Furminger, 1963) and there was apparently a complete absence of particles from the many control cultures examined. The particles appeared following inoculation of the virus and their numbers seemed to increase pari passu with the increase in infective virus in the culture. However, as it no longer seems possible to regard the particles described as being rubella virus, the possibility of their being a mycoplasma has been re-examined.

Full details of this work will appear elsewhere. The purpose of this brief note is simply to establish the identical morphology of the particles described by Chatterji et al. (1969) and those of a mycoplasma isolated from lines of RK 13 cells used in Australia for the isolation and propagation of rubella virus. Both the cells themselves and the supernatant fluids from the Australian cultures yielded colonies of a mycoplasma when plated on solid medium and incubated anaerobically (Cross, Goodman & Shaw, 1967). The organisms isolated reacted in various tests with antisera to both Mycoplasma orale Type 1 and Mycoplasma fermentans. The line of RK 13 cells used by Chatterji et al. was recently retested and yielded M. orale, Type 1. The identity of the Australian isolates is still being studied.

The Australian RK 13 cells could be readily freed from the contaminating mycoplasma by treatment for 48 to 72 hr with sodium aurothiomalate 0.1 mg/ml. (Cross et al. 1967). During 51 subsequent passages without the drug the treated cells showed no evidence of mycoplasma infection as judged by direct culture, the indirect fluorescent antibody technique and electron microscopy.

Stocks of rubella virus (strain BAYLOR) were inoculated into untreated cells and into treated cells. In the latter case, sodium aurothiomalate was added to the maintenance medium to deal with mycoplasma in the inoculum. The titres of the virus and the final virus yields were identical in the two systems. Once virus stock and cells both free from demonstrable mycoplasma had been obtained, the virus could be propagated in the absence of the gold salt. During 15 such passages, no evidence of mycoplasma infection was detected in either cells or culture fluid. The virus behaved normally and, in particular, produced the normal cytopathic effect. There was thus no evidence to indicate that the presence of the mycoplasma in RK 13 cells influenced the replication of rubella virus one way or the other. There was also nothing to suggest that the sodium aurothiomalate affected either the cells or the virus adversely.
As in the earlier study, cells for electron microscopy were fixed in glutaraldehyde in situ, post-fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Fig. 1 is taken from Chatterji et al. (1969) (their Plate 3) and shows some of the types of particle which they described. Fig. 2 shows part of the surface of an untreated Australian RK13 cell and Fig. 3 parts of two cells from a Hep-2 culture deliberately infected with *M. orale*,

Fig. 1. Cells fixed in glutaraldehyde, post-fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Taken from Chatterji et al. (1969), their Plate 3. RK13 cells inoculated with rubella, strain 171, 36 hr earlier.
Type 1. Neither of the cultures depicted in Figs. 2 and 3 had been exposed to rubella virus. There can be no question that the particles shown in all three figures are virtually identical and, therefore, that at least the majority of the particles seen by Chatterji et al. were of mycoplasma and not of rubella virus. The small number of particles which they observed to

Fig. 2. Cells fixed in glutaraldehyde, post-fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Untreated Australian RK13 cells showing cells of mycoplasma.
Fig. 3. Cells fixed in glutaraldehyde, post-fixed in osmium tetroxide and stained with uranyl acetate and lead citrate. Hep-2 cells deliberately infected with *Mycoplasma orale*, Type 1.
have the ring-like appearance and the central nucleoid may have been particles of rubella virus (Fig. 1).

The failure of Chatterji et al. to observe similar particles in the large amount of control material examined is difficult to explain, as both the strains of virus which they used had been passed in the same line of RK 13 cells just before the experiments which they describe and one of the strains, 171, had only been passed in these cells since it was isolated. This, together with the observation that many of the virus harvests from the untreated Australian cells show higher infectivity titres of mycoplasma than of virus, must raise the possibility that even though the mycoplasma does not influence the virus, the converse might not always be true. However, studies to date have not indicated any difference between titres of mycoplasma attained in rubella-infected and control cultures.

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REFERENCES


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