

Rubin H. A virus in chick embryos which induces resistance *in vitro* to infection with Rous sarcoma virus. *Proc. Nat. Acad. Sci. US* 46:1105-19, 1960.
[Department of Virology and Virus Laboratory, University of California, Berkeley, CA]

Occasional cell cultures made from chicken embryos were resistant to infection with Rous sarcoma virus (RSV). A virus was isolated from the medium of these cultures that caused sensitive cultures to become resistant. This virus belonged to the avian lymphomatosis group. Other viruses of the group also induced resistance to infection by RSV. This provided the first assay *in vitro* for leukemia viruses. [The *SCI*® indicates that this paper has been cited in over 410 publications since 1960.]

Harry Rubin
Department of Molecular Biology
University of California
Berkeley, CA 94720

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This paper describes the solution to a problem that had been frustrating my study of Rous sarcoma virus (RSV) in cell culture for several years. It seemed that whenever I would work up to doing a large experiment, I would find out *ex post facto* that the cultures I had expended so much effort on were resistant to RSV infection. I tried every physiological variation I could think of to avoid the resistance problem but to no avail. Actually, in those weeks that I set up variations to study the resistance, the chick embryo cell cultures would be fully sensitive to RSV—a little like carrying an umbrella, which

seems to prevent rain. Finally, I decided that if the resistance occurred again, I would drop whatever I was doing and keep the resistant cultures going for further study. Finally, lightning struck, and, on an off chance, I took some of the medium from a resistant culture and added it to a sensitive culture. Lo and behold, after a few days, the sensitive culture became resistant.

To make a long story short, it turned out that an occasional chick embryo was congenitally infected with a retrovirus, which produced no recognizable pathology in the cells. When the cells of this embryo were pooled with the cells of other embryos, as was then our practice, the others became infected and resistant to RSV. I named the virus RIF for resistance inducing factor, but further work proved it was an avian leukosis virus, as I indicated in the paper.

By infecting sensitive cultures with serial dilutions of RIF and determining which became resistant to RSV, I could assay for RIF. This proved to be the first assay in cell culture for a virus of the leukemia-causing class. However, I do not think that is why the paper has been frequently cited, since the RIF assay has not been much used. Rather, I think it is cited because I included a fairly complete description of the assay for RSV. Since work with RSV became very popular, I suspect many people needed the latest description of its biological assay and, therefore, cited my paper. That is too bad, because the paper had a lot of interesting stuff about leukosis viruses, including their congenital transmission. In fact, it made it possible to eliminate the leukosis virus from an experimental flock of chickens.¹ It also made possible the discovery of the defectiveness of RSV.² But...that's the way it goes.

1. Hughes W F, Watanabe D H & Rubin H. The development of a chicken flock apparently free of leukosis virus. *Avian Dis.* 7:154-65, 1963. (Cited 30 times.)
2. Hanafusa H, Hanafusa T & Rubin H. The defectiveness of Rous sarcoma virus. *Proc. Nat. Acad. Sci. US* 49:572-80, 1963. (Cited 265 times.)