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Temin H M & Rubin H. Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. *Virology* 6:669-88, 1958.
[Division of Biology, California Institute of Technology, Pasadena, CA]

This paper describes the first quantitative and reproducible cell culture assay for neoplastic transformation by Rous sarcoma virus, a highly oncogenic retrovirus. [The *SCI*® indicates that this paper has been cited in over 530 publications.]

Neoplastic Transformation in Cell Culture

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Virus-caused cancers were first found by V. Ellermann and O. Bang and by Peyton Rous in the first decade of this century.[1,2] However, by the middle 1950s, viral causation of cancer was not considered to have any connection with cancer in general, and the study of tumor viruses was conducted in only a few laboratories. The present connection between viral carcinogenesis and genetic theories of cancer resulted from the study of transformation of cells in culture.

Rous sarcoma virus (RSV) was the first virus accepted as a cause of cancer, although avian erythroblastosis virus and the precursor to avian myeloblastosis virus had been isolated earlier. RSV was first assayed by its ability to form tumors in chickens and then by its ability to form pocks on the chorioallantoic membrane of the developing chicken embryo. In the late 1940s and early 1950s, cell culture was developed as a quantitative tool for animal virology. R. Dulbecco developed a plaque assay for Western equine encephalitis virus in tissue culture, starting quantitative cell culture animal virology.[3] But carcinogenesis and neoplastic transformation were studied only in living animals.

I was a graduate student in embryology at the California Institute of Technology with Albert Tyler, studying the artificial activation of eggs of *Urechis caupo* and acting as a laboratory assistant in the elementary biology course taught by George Beadle. I was assigned to obtain fertilized chicken eggs from Dulbecco's laboratory. Harry Rubin, a postdoctoral fellow in that laboratory, supplied me with the eggs and told me about chicken sarcoma viruses isolated decades earlier by Rous. Rubin especially described chicken sarcoma viruses numbers 9 and 10, which induced bone tumors in infected muscle. He also described a paper he was refereeing for *Virology* by R.A. Manaker and V. Groupé on morphological alterations in cells infected with RSV.[4] Rubin suggested that I try to make a quantitative tissue culture assay based on their observations.

Soon thereafter I switched from embryology to become a graduate student in virology with Dulbecco, and I set out to develop a quantitative cell culture assay for RSV under Rubin's direction. At that time cell culture was rather primitive, and we grew cells in small glass Carrel flasks. Our stocks of RSV were tumor homogenates supplied by Ray Bryan of the National Cancer Institute, and they were assayed on the chorioallantoic membrane of the chicken embryo. The breakthrough in making the cell culture assay for RSV came when I decided not to plate the chicken embryo cells at a density that would form a monolayer, as was done for all the previous cell culture animal virus assays, but to plate them to form a sparse layer of cells. When RSV was applied to this sparse cell layer and the cells were subsequently allowed to divide, foci of morphologically transformed cells appeared in numbers proportional to the concentration of the virus.

This was the first quantitative assay for neoplastic transformation *in vitro*. It established that RSV by itself could transform cells and that it transformed fibroblastic cells. This assay was followed by similar cell culture assays for transformation by polyoma virus and other DNA tumor viruses and then for transformation by chemicals and irradiation. The cell culture assays opened the way to the research that has now defined reverse transcription, oncogenes, and proto-oncogenes and has shown the fundamental connections between retroviral carcinogenesis and all other carcinogenesis.[5]

1. **Ellermann V & Bang O.** Experimentelle Leukämie bei Hühnern (Experimental leukemia in chickens). *Zentralbl. Bakteriol. Parasitenkd. Infektionskrankh. Hyg. Abt. I Ref.* 46:595-609, 1908. (Cited 215 times since 1945.)
2. **Rous P.** A sarcoma of the fowl transmissible by an agent separable from the tumor cells. *J. Exp. Med.* 13:397-417, 1911. (Cited 405 times since 1945.)
3. **Dulbecco R.** Production of plaques in monolayer tissue culture by single particles of an animal virus. *Proc. Nat. Acad. Sci. USA* 38:747-52, 1952. (Cited 540 times.)
4. **Manaker R A & Groupé V.** Discrete foci of altered chicken embryo cells associated with Rous sarcoma virus in tissue culture. *Virology* 2:838-40, 1956. (Cited 130 times.)
5. **Bishop J M.** The molecular genetics of cancer. *Science* 235:305-11, 1987. (Cited 290 times.)

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