CC/NUMBER 33
AUGUST 17, 1981

This Week's Citation Classic

Hirt B. Selective extraction of polyoma DNA from infected mouse cell cultures. *J. Mol. Biol.* **26**:365-9, 1967.
[Swiss Inst. Experimental Cancer Res., Lausanne, Switzerland]

Cells infected by polyoma virus are lysed by the addition of the detergent sodium dodecyl sulfate. The detergent can be precipitated with sodium chloride at 4°. The DNA of the cells, if not sheared, precipitates too. The viral DNA however, which is smaller in size by several orders of magnitude, stays in the supernatant. [The *SCI*® indicates that this paper has been cited over 1,135 times since 1967.]

Bernhard Hirt
Swiss Institute for Experimental
Cancer Research
CH-1066 Epalinges
Switzerland

July 15, 1981

"In the fall of 1964, Roger Weil accepted me in his laboratory, where with two co-workers, he was studying the interaction of polyoma virus with its host cells Before, I had been working in physics, so everything was new and exciting to me. I was assigned to the task of studying the replication of the viral DNA in cultured cells. From Weil's[1] work I knew that viral DNA is synthesized simultaneously with the DNA of the host, a complicating fact.

"First, I tried to inhibit preferentially the host DNA synthesis by irradiating the cells with x-rays or ultraviolet light prior to infection. But the replication of the viral DNA was inhibited too. I then looked for ways to separate viral DNA from cellular DNA. In their study, Weil, Michel, and Ruschmann[1] had extracted the DNA by a method which in principle had already been used by Avery[2] and co-workers in their historical study: cells were lysed with detergent and the viscosity of the lysate was reduced by pipetting and shaking. The detergent was then precipitated with salt, leaving a mixture of host cellular and viral DNA in the supernatant. Sedimentation velocity analysis in a sucrose gradient showed a fast-sedimenting band of host DNA with a peak of viral DNA on the slow side. For my purpose the resolution of the two DNAs was not sufficient. I wanted the host cellular DNA to sediment faster. I therefore avoided pipetting during the extraction, because shearing forces were known to reduce the molecular weight and the sedimentation coefficient of the DNA. In the following sucrose gradient sedimentation I expected to find a fast-sedimenting peak of cellular DNA, well separated from the slow viral peak. At 11 pm one night in October 1965 I put the samples into the counter and realized after a few minutes that the cellular DNA was not there. I concluded that the experiment had failed, said a word that in English would take four letters and went home to sleep. The rest of the samples had been counted by the time I arrived the next morning and I jumped up when I realized that the modified extraction had produced pure viral DNA. The extraction method was of use immediately in a study on semiconservative replication of polyoma DNA.[3]

"Later I wrote down the procedure and sent it as a letter to the editor of the *Journal of Molecular Biology*. One referee worked hard on it and sent a report three pages long, typed single-spaced, with many suggestions and requests for additional data. Despite some good remarks, the report made me angry by its paternalistic tone (I didn't realize at that time that refereeing papers is not always fun). My manuscript went into a drawer for two and a half months until an American postdoc, David A. Goldstein, came to our lab and helped me rewrite it. I assured the editor that the comments of the referee had been taken into account and the paper was published with no delay.

"The extraction method is simple and effective for isolating small DNA molecules from both bacteria and animal cells. This, together with the fact that many researchers chose to study small DNA viruses, might explain the high number of citations. More recent work in the field is reported by R.A. Weinberg."[4]

1. **Weil R, Michel MR & Ruschmann G K.** Induction of cellular DNA synthesis by polyoma virus. *Proc. Nat. Acad. Sci. US* **53**:1468-75, 1965.
2. **Avery O T, MacLeod C M & McCarty M.** Studies on the chemical nature of the substance inducing transformation of pneumococcal types. *J. Exp. Med.* **79**:137-58, 1944.
3. **Hirt B.** Evidence of semiconservative replication of circular polyoma DNA. *Proc. Nat. Acad. Sci. US* **55**:997-1004, 1966.
4. **Weinberg R A.** Integrated genomes of animal viruses. *Annu. Rev. Biochem.* **49**:197-226, 1980.