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Calnek B W, Adldinger H K & Kahn D E. Feather follicle epithelium: a source of enveloped and infectious cell-free herpesvirus from Marek's disease. *Avian Dis.* 14:219-33, 1970.
[Depts. Avian Diseases and Microbiology, New York State Veterinary Coll., Ithaca, NY]

Marek's disease herpesvirus, which causes lymphomas in chickens, is highly cell-associated. Keratinized cells surrounding the feather shafts in the skin were found to be unique in their production of enveloped virions, which could be extracted in infectious form and used cell-free to reproduce the disease in chickens. [The *SCI*® indicates that this paper has been cited in over 145 publications since 1970.]

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"Excitement in the area of avian tumor research, especially that concerning Marek's disease (MD), was running very high in the 1960s, and so it was only natural that I should join the throng as a young scientist returning 'home' to Cornell University. MD was by far the most serious disease of chickens (annual cost in the US of nearly 200 million dollars), and it appeared to be a good model for viral oncogenicity studies.

"In 1967, the etiology was determined by others to be a highly cell-associated herpesvirus,[1,2] just as I was going off to the University of California at Berkeley for a sabbatical leave. Thus, it was not until my return in 1968 that I could take full advantage of this finding. During my absence, J. Lloyd Spencer, my graduate student, had developed an immunofluorescence test for localizing viral antigen in tissues. His procedures offered an opportunity to address some very intriguing questions regarding the pathogenesis of MD. Because he had finished his studies, I enlisted the help of S.B. Hitchner, and we constructed a study in which 43 separate tissues involving all organ systems were examined sequentially for viral antigen over a period of several weeks. We purposely included tissues from which virus could gain access to the environment, since one of the most puzzling questions about MD was how it could spread so rapidly within a flock when the agent was so highly cell-associated.

"The skin sections included embedded feather shafts, and it was the feather follicle epithelium (FFE) that took top honors in terms of frequency of infection and amount of viral antigen. Obviously, that tissue offered a plausible location for virus shedding, since molted feathers or ordinary dander would include desquamated keratinized cells from the FFE. This study was published in the *Journal of the National Cancer Institute* in 1969, and we duly noted the possible significance of the FFE.[3]

"The next steps were easy. With the help of two graduate students (Hans Adldinger and Donald Kahn), cell-free skin extracts were shown to be highly infectious and able to induce MD in chickens. Electron microscopic examination proved the existence of enveloped herpes virions. The work was relatively simple and straightforward once we had stumbled onto the possible significance of the FFE. The difficulties, instead, had to do with acceptance of its validity. Perhaps no one thought that the paradox of a cell-associated herpesvirus that could be easily spread should be so simply solved. Fortunately, colleagues abroad, who I believe initially mistrusted the data, easily confirmed it, as did colleagues at home, who through a curious and poorly understood (by me) set of circumstances managed to prepublish us.[4] Regardless, it constituted one of the most thrilling periods of my scientific career and probably gained me more attention than anything else I had done to that time.

"It is probable that frequent citation is attributable to two factors. First, the paper offered the methodology required to obtain cell-free MD virus. Second, the use of that methodology was widespread and prolonged in the very active field of MD research."[5,6]

1. **Churchill A E & Biggs P M.** Agent of Marek's disease in tissue culture. *Nature* 215:528-30, 1967. (Cited 335 times.)
2. **Nazerian K, Solomon J J, Witter R L & Burmester B R.** Studies on the etiology of Marek's disease. II. Finding of a herpesvirus in cell culture. *Proc. Soc. Exp. Biol. Med.* 127:177-82, 1968. (Cited 210 times.)
3. **Calnek B W & Hitchner S B.** Localization of viral antigen in chickens infected with Marek's disease herpesvirus. *J. Nat. Cancer Inst.* 43:935-49, 1969. (Cited 140 times.)
4. **Nazerian K & Witter R L.** Cell-free transmission and in vivo replication of Marek's disease virus (MDV). *J. Virology* 5:388-97, 1970. (Cited 125 times.)
5. **Payne L N, Frazier J A & Powell P C.** Pathogenesis of Marek's disease. *Int. Rev. Exp. Pathol.* 16:59-154, 1976. (Cited 80 times.)
6. **Calnek B W.** Marek's disease virus and lymphoma. (Rapp F, ed.) *Oncogenic herpesviruses.* Boca Raton, FL: CRC Press, 1980. p. 103-43.

