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This Week's Citation Classic®

Bachrach H L, Trautman R & Breese S S. Chemical and physical properties of virtually pure foot-and-mouth disease virus. *Amer. J. Vet. Res.* 25:333-42, 1964.
[Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, ARS, USDA, Greenport, Long Island, NY]

Foot-and-mouth disease (FMD) virus, type A_{12}, was produced in essentially pure form and analyzed for sedimentation rate (140 S), EM diameter (23mμ), specific infectivity (10^{11} plaque-forming units [PFU]/ml), virions ($10^{13.6}$/ml), chemical composition (31.5 percent RNA/68.5 percent protein), absorbance spectrum (A_{max} = 259 mμ), extinction coefficient ($E^{1\%}_{259\ m\mu} \cong 76$), and specific refractive increment (0.158 ml/gm). Viral RNA had a T_m of 55° C and mole fractions of bases as follows: G = 0.24; A = 0.26; C = 0.28; and U = 0.22. [The *SCI*® indicates that this paper has been cited in over 145 publications.]

Howard L. Bachrach
Plum Island Animal Disease Center
US Department of Agriculture
Greenport, NY 11944-0848

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While establishing the sedimentation rate for FMD virus (~140 S) in Denmark in 1949,[1] I sometimes daydreamed that the outermost viral proteins, after isolation from the virus, would induce protective immunity in livestock. After a stint (1950-1953) purifying poliovirus in Wendell Stanley's virus lab at the University of California, Berkeley, the opportunity to work on the daydream presented itself in 1953, when the US Department of Agriculture hired me to organize the biochemical investigations section at their new Plum Island research facility for foreign animal diseases.

The *Citation Classic* on virtually pure FMD virus is one of many stepping-stones needed to reach the goal of a protein subunit vaccine. It's still often cited for use of $E^{1\%}_{259\ m\mu} \cong 76$ in calculating the concentration of purified FMD virus preparations. Subsequent stepping-stones included the production and purification of virus in 100-mg and larger amounts from cell cultures, the demonstration of 4 different proteins (60 copies of each) in the FMD virus coat, and the preparative separation and recovery of these coat proteins on large hollow-cylindrical SDS/8 M urea polyacrylamide gels. Our progress was facilitated greatly by finding that the separated protein bands could be visualized immediately after electrophoresis by simply chilling the gels for 5 to 10 minutes.[2] In 1975 we reported that of the four proteins, only the 24 kD protein (213 amino acids) would induce neutralizing and virus-precipitating antibodies in guinea pigs.[3] We also reached the real-world goal of being the first to show that the 24 kD protein would induce protective immunity in swine, and later proved that a 13 kD fragment (residues 55 to 179) excised from the 24 kD protein using CNBr would do the same.

By 1977, however, recombinant DNA was becoming the "in" thing, allowing us another dream—cloning and expressing the gene for the 24 kD protein in *Escherichia coli*. After identifying 24 kD-specific gene (redundant) sequences by sequencing parts of the 24 kD protein and having discussed our intention to clone the 24 kD-specific gene in a publication,[4] we accepted Dennis Kleid's (Genentech, Inc.) offer of collaboration in late 1979. By 1981 we reported in *Science*[5] on the immunization of cattle and swine with a 44 kD fusion protein containing 206 of the 213 amino acid residues of the 24 kD viral coat protein. This publication was awarded the AAAS-Newcomb Cleveland Prize (in competition across all fields of science), and US Secretary of Agriculture John R. Block called it "the first production through gene splicing of an effective vaccine against any disease in animals or humans." [Editor's note: This paper also led to membership in the National Academy of Sciences and the award of the National Medal of Science.]

1. **Bachrach H L.** The determination of the sedimentation constant of a homogeneous component having the characteristics of the foot-and-mouth disease virus. *Amer. J. Vet. Res.* 13:13-6, 1952.
2. ————. Visualization by chilling of protein bands in polyacrylamide gels containing 8 molar urea: preparation and quantitation of foot-and-mouth disease virus capsid proteins. *Anal. Biochem.* 110:349-54, 1981.
3. **Bachrach H L, Moore D M, McKercher P D & Polatnick J.** Immune and antibody responses to an isolated capsid protein of foot-and-mouth disease virus. *J. Immunology* 115:1636-41, 1975. (Cited 115 times.)
4. ————. An experimental protein vaccine for foot-and-mouth disease. (Pollard M, ed.) *Gustav Stern Symposium on Perspectives in Virology*. New York: Raven Press, 1978. Vol. 10. p. 147-59. (Cited 15 times.)
5. **Kleid D G, Yansura D, Small B, Dowbenko D, Moore D M, Grubman M J, McKercher P D, Morgan D O, Robertson B H & Bachrach H L.** Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and swine. *Science* 214:1125-9, 1981. (Cited 150 times.)

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