

This Week's Citation Classic

Fraenkel-Conrat H, Singer B & Tsugita A. Purification of viral RNA by means of bentonite. *Virology* 14:54-8, 1961. [Virus Laboratory, University of California, Berkeley, CA]

This paper represents the less important half of a piece of work published as two papers (p. 54 and 59). It demonstrates the efficacy of bentonite in diminishing residual traces of peptides in phenol-prepared viral RNA. The conclusion is that the upper limit for tobacco mosaic virus (TMV) protein contamination in such RNA preparations was one mole per 23 moles of the RNA. [The *SCI*[®] indicates that this paper has been cited over 500 times since 1961.]

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"The fact that this paper is cited with sufficient frequency to warrant its inclusion in this series is an illustration of the fallibility of citation frequency as a measure of the importance of a piece of work. All three authors have at least 10 other papers to their credit which they would list above this one in importance. And what this paper is quoted for is not its intrinsic point (which had some importance), but the fact that it contains a paragraph describing the method of washing and suspending the commercial bentonite clay. The purpose of preparing such bentonite suspensions is described in the companion paper: the adsorption and removal of ribonucleases leads to considerably more stable RNA preparations, and when tested in presence of bentonite TMV RNA shows much higher infectivity.¹ It is these findings by Singer and Fraenkel-Conrat which account for the popularity of this paper!

"What led to the research reported in these papers? As so frequently, and as it should be, it was a report in the literature seemingly unrelated to our work and yet of potential usefulness, namely that the clay,

bentonite, binds and inhibits ribonucleases. Bea Singer and I had, since 1956, become convinced that the infectivity and thus the entire genetic information of TMV resided in its RNA.^{2,3} (It is these papers one would expect to be most frequently cited, since TMV RNA was the first physico-chemically definable molecular species shown to represent a genome.) These studies were difficult because of the great sensitivity of RNA to the ubiquitous ribonucleases and the requirement of the intact molecule of 6390 nucleotides for TMV infectivity. Thus, not surprisingly, we were on the look-out for anti-nuclease agents and tested all that came to our attention. And of these, bentonite proved the most useful, as described above, notwithstanding the first reaction of a colleague also working with TMV RNA at Berkeley, 'I certainly would not put this mud into my RNA preparations.'

"On the basis of these findings it seemed only logical that removal of traces of ribonucleases by bentonite should cause a decrease in the 0.5-1 % of protein seemingly present in such viral RNA preparations, and still representing a last hope of the die-hards who disbelieved RNA's potency. We were successful. Upon degrading ³⁵S labelled TMV in presence of bentonite, the amount of ³⁵S in the viral RNA was reduced by one order of magnitude. Also, Tsugita's analyses showed the amino acid composition of the residual 0.04% of protein(s) or peptide(s) to be quite unlike that of TMV protein. The finding that the residual trace of ³⁵S was displaced by reconstituting such RNA with TMV protein further proved that it did not represent residual virus or viral protein. Thus the much-quoted paper represented one more proof (than was needed) that viral RNA molecules represent fully competent genomes.

"B. Singer is currently at the Virus Laboratory, University of California, Berkeley, CA and A. Tsugita is at the EMBO Laboratory, Heidelberg, Federal Republic of Germany."

1. Singer B & Fraenkel-Conrat H. Effects of bentonite on infectivity and stability of TMV-RNA. *Virology* 14:59-65, 1961.
2. Fraenkel-Conrat H. The role of the nucleic acid in the reconstitution of active tobacco mosaic virus. *J Amer. Chem. Soc.* 78:882-3, 1956.
3. Fraenkel-Conrat H, Singer B & Williams R C. Infectivity of viral nucleic acid. *Biochim. Biophys. Acta* 25:87-96, 1957.