

# This Week's Citation Classic

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**Bryant M P & Robinson I M.** An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. *J. Dairy Sci.* **44**:1446-56, 1961.  
[Animal Husbandry Res. Div., Agricultural Res. Serv., US Dept. Agriculture, Beltsville, MD]

**An agar roll-tube medium doubled the viable count of fermentative bacteria and made colony counting and isolation of strains easier. The improvement was due to a small amount of Na<sub>2</sub>S added to the cysteine reducing solution, very small amounts of energy source, and clarified rumen fluid. [The SC<sup>i</sup>® indicates that this paper has been cited over 190 times since 1961.]**

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"The research was initiated to explore possibilities (and using statistical analyses) for improvement of the rumen fluid-glucose-cellobiose-agar (RGCA) medium for isolation and enumeration of strains of rumen bacteria, and also to prove that numbers of viable bacteria in the rumen of cattle varied significantly in relationship to animal, diet, and time after feeding. Use of the roll-tube, habitat-simulating medium and methods slightly modified from those of R.E. Hungate combined with an anaerobic balanced mineral diluting solution had already proved to be best for rumen bacteria. However, many labs persisted in using culture media containing large amounts of materials such as yeast and meat extracts, casein digests and carbohydrate energy sources, and enumeration methods exhibiting as much as 100-fold errors due to methods. Whereas due mainly to direct microscopic counts, we already knew that numbers usually varied less than 10-fold.

The RGCA medium contained .2 percent each of glucose and cellobiose which resulted in very large colony formation and spreading such that enumeration and isolation of strains were more difficult, especially if incubations were extended beyond the minimal three days (39°C) and starch or maltose required by a few bacterial species was not included. Thus, additional work to improve the rumen fluid-based medium was warranted.

"The paper is probably cited for many reasons, a number of which became apparent later. Among the better reasons were the greatly expanded interest in anaerobic bacteria brought about by Hungate and his students (I was his first in rumen microbiology); the use of similar methods on fermentative anaerobes often of medical interest, e.g., the work of W.E.C. Moore<sup>1</sup> and associates at the Anaerobe Lab, Virginia Polytechnic Institute; the use of similar methods for methanogens first by Hungate and his students (especially P.H. Smith<sup>2</sup>) and by Robinson and me<sup>3</sup> and then by my University of Illinois colleague R.S. Wolfe and his students;<sup>4</sup> and the use in the first studies on syntrophy between H<sub>2</sub>-using methanogens and ethanol or fatty-acid catabolizing, acetate and H<sub>2</sub>-producing bacteria<sup>5,6</sup> which led to the concept of interspecies H<sub>2</sub> transfer—facilitated by my colleague, M.J. Wolin<sup>7</sup> and several of his students.

"The double reducing system of cysteine and sulfide and the manner of its use in the study turned out to be decisive in studies on methanogens. This included studies with Robinson<sup>5</sup> on the nutrition of *Methanobrevibacter ruminantium* involving essentiality of NH<sub>4</sub><sup>+</sup> as main N source, acetate and 2-methylbutyrate as essential carbon sources and another unidentified rumen fluid factor. This was later chemically identified by Wolfe and his students as the first of several coenzymes present in all methanogens but not in other life forms."<sup>4</sup>

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3. Bryant M P, Tzeng S F, Robinson I M & Joyner A E. *Advan. Chem.* **105**:23-40, 1971.
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