

This Week's Citation Classic®

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Caldwell D R & Bryant M P. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Appl. Microbiol.* 14:794-801, 1966. [Animal Husbandry, Research Division, US Department of Agriculture, Beltsville, MD]

Colony counts that approximated those in a habitat-simulating rumen fluid-agar medium (RFM) were obtained in medium 10, a medium identical to the RFM except for the replacement of rumen fluid with 1.5×10^{-6} M hemin, 0.2 percent Trypticase, 0.05 percent yeast extract, and a 6.6×10^{-2} M volatile fatty acid mixture qualitatively and quantitatively similar to that of rumen fluid. [The SC⁹ indicates that this paper has been cited in over 240 publications.]

Daniel R. Caldwell
Department of Molecular Biology
University of Wyoming
Laramie, WY 82071
and
Marvin P. Bryant
Department of Animal Sciences
University of Illinois
Urbana, IL 61801

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The decade between 1960 and 1970 was a period of increasing interest in the study of nonpathogenic, strictly anaerobic bacteria, particularly those found in the gastrointestinal tract of warm-blooded animals.¹ Although interest in the study of gut anaerobes was, and continues to be, intense,^{2,3} difficulties were encountered initially in their cultivation, primarily because early workers often used traditional culture media. As knowledge concerning gut microbes increased, the importance of habitat simulation to the isolation of anaerobes from natural environments became apparent. It was recognized that habitat-simulating media provided essential unusual growth factors, for example, branched-chain volatile fatty acids, carbon dioxide, and heme, which were required by gut anaerobes and which were absent in conventional media.⁴

Much of the early work with gastrointestinal anaerobes involved rumen bacteria, but, after a period of time, it was recognized that organisms physiologically and biochemically similar to rumen bacteria were found in appropriate anatomical locations in the gut of a variety of warm-blooded animals. This recognition spurred efforts to devise media suitable

for the cultivation and isolation of a wide variety of gut anaerobes.

In 1953 Marvin P. Bryant and L.A. Burkey developed a rumen fluid, glucose cellobiose agar (RGCA) medium. The medium contained 40 percent rumen fluid and was a relatively nonselective medium for isolation of anaerobes.⁵ The percentage of rumen fluid used was arrived at after careful study and was used because it allowed growth of both the maximum number and greatest species diversity of rumen organisms. The medium was modified in 1961.⁶

The development of medium 10, the subject of this *Citation Classic*, was the result of an effort to devise a nonrumen-fluid medium that nutritionally and physiochemically simulated the gut habitat without the necessity of rumen fluid, since the latter is not always available to those who would study gut bacteria. Furthermore, rumen fluid is not a substance likely to be found in perfumes and is not particularly conducive to retaining one's breakfast. Medium 10 was designed to contain nutrients and growth factors similar to those found in the RGCA medium without the use of rumen fluid. The final formulation of medium 10 was determined after careful study regarding both the sources of its ingredients and their concentration.

The efficacy of medium 10 for nonselective enumeration and isolation of rumen bacteria was assessed by comparing the number and species distribution of organisms randomly isolated from diluted rumen contents obtained from animals fed a variety of diets and inoculated into medium 10, with the analogous parameters in the same samples inoculated into modified RGCA medium. In this process over 1,200 isolates were obtained and presumptively identified.

During the time of the study funding for our group at Beltsville, Maryland, excluding salaries, was about \$4,000 for everything—supplies, equipment, publication costs, travel, and so on for four scientists. To conserve funds we used the same animals, in some cases, for studies of the effects of different diets. In the process of changing diets, the animals almost died.

The usefulness of medium 10 reflects the common physiology and biochemistry of gastrointestinal anaerobes. Although the medium is not suitable for all purposes, it is my hope that it will be useful to anaerobic microbiologists for many years to come.

1. Hungate R E. *The rumen and its microbes*. New York: Academic Press, 1966. 533 p. (Cited 1,110 times.)
2. Clarke R T J & Bauchop T, eds. *The microbial ecology of the gut*. New York: Academic Press, 1977. 410 p.
3. Hentges D, ed. *Human intestinal microflora in health and disease*. New York: Academic Press, 1983. 568 p.
4. Bryant M P & Robinson I M. Some nutritional characteristics of predominant cultural ruminal bacteria. *J. Bacteriology* 84:605-14, 1962. (Cited 175 times.)
5. Bryant M P & Burkey L A. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. *J. Dairy Sci.* 36:205-17, 1953. (Cited 330 times since 1955.)
6. 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. (Cited 250 times.)