A medium of powdered bovine albumin supplemented with polysorbate 80 (Tween 80), and with NH₄Cl, vitamin B₁₂, thiamine, and trace metals, replaced whole rabbit serum, supporting continuous leptospiral growth. [The SCI® indicates that this paper has been cited in over 130 publications.]

**This Week's Citation Classic**


**Nutrients for a Spirochete**

Herman C. Ellinghausen
Ellinghausen Associates
105 Spa View Avenue
Annapolis, MD 21401

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In 1955 the veterinary research laboratory of the US Department of Agriculture, devoted to the study of bacterial diseases of domestic animals, initiated a leptospirosis research project. One goal was to study nutrition, growth, cultural characteristics, isolation, pathogenicity, serology, and immunology of these, the most cultivable of the spirochetes.

My studies, begun in 1955, resulted in development of a liquid medium, consisting of bovine albumin, NH₄Cl, polysorbate 80, thiamine, and vitamin B₁₂. Addition of agar at 0.2 percent and 1 percent resulted in a stock culture and isolation medium and a plating medium for colonial growth.

Since the discovery of these spirochetes in 1915, rabbit serum had been a mandatory additive to growth media. Growth measurements such as optical density, cell counts under darkfield, dry weight, and cellular nitrogen, proved impractical. The happenstance finding of a Coleman-7 nephelometer (without filters) in our attic was to become a keystone to my future progress. No one knew how to use the instrument, or even standardize it.

Dr. Bill Roessler's inert titanium dioxide was found superior to standardize the nephelometer. Using rabbit serum to support growth, I researched culture variables and found a linearity to growth over a specific concentration of serum.

Then, in 1960, while lecturing to a group of students on the lipid composition of bacteria, I commented that anyone useful in growing mycobacteria should aid in growing leptospires.

Limited by using rabbit albumin for large-scale propagation, A.M. Schneiderman et al.¹ had turned to crystalline bovine albumin. They overlooked the need for lipid and vitamin B₁₂ and, consequently, their studies failed.

Considering the broad composition of rabbit serum (proteins, carbohydrates, salts, vitamins, and bound lipids), in 1960 we exhaustively dialyzed rabbit serum under aseptic conditions against sterile distilled water. We realized that with carbohydrates gone and protein still present, growth was possible. In 1949 P.B. Marshall² identified rabbit serum as a respiratory stimulant using the Warburg. Data by J.O. Fulton and D.F. Spooner³ and J.J. Helprin and C.W. Hiatt⁴ confirmed we were dealing with the oxidation of fatty acids.

B. Babudieri's supplementation of nutrient deficient serum with vitamin B₁₂,¹ and Schneiderman's failure with crystalline bovine albumin¹ led to the conclusion that vitamin B₁₂ was needed. Oleic albumin complex in medium with NH₄Cl, vitamin B₁₂, and thiamine worked. Reconstitution of the lipid extracted albumin with oleic acid was successful. Polysorbate 80 (Tween 80), a water soluble heat autoclavable fatty acid source, made the medium practical.

When our findings were submitted to a major bacteriological journal, the work was rejected as insignificant and lacking originality since, 15 years earlier, bovine albumin had been used in studies.

Using the medium, the first isolation of *Leptospira grippotyphosa* from cattle in the US was achieved in 1964. Encouraged by this, we submitted our work to a veterinary journal; the findings were judged a breakthrough.

In time, practically all leading leptospiral bacteriologists, switched from using bovine albumin polysorbate 80 medium. Great progress was made in the isolation of *L. hardjo,*⁶ therefore almost uncultivable.

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