This Week's Citation Classic®

Ellinghausen H C & McCullough W G. Nutrition of *Leptospira pomona* and growth of 13 other serotypes: fractionation of oleic albumin complex and a medium of bovine albumin and polysorbate 80. *Amer. J. Vet. Res.* 26:45-51, 1965.
[Bacteriological and Chemical and Physical Investigations, Natl. Animal Disease Lab., Animal Disease and Parasite Res. Div., ARS, USDA, Ames, IA]

A medium of powdered bovine albumin supplemented with polysorbate 80 (Tween 80), and with NH_4Cl, vitamin B_{12}, 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.]

Nutrients for a Spirochete

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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 the 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, NH_4Cl, polysorbate 80, thiamine, and vitamin B_{12}. 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 anything 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 B_{12} 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. Marshall[2] had identified rabbit serum as a respiratory stimulant using the Warburg. Data by J.D. Fulton and D.F. Spooner[3] and J.J. Helprin and C.W. Hiatt[4] confirmed we were dealing with the oxidation of fatty acids.

B. Babudieri's supplementation of nutrient deficient serum with vitamin B_{12}[5] and Schneiderman's failure with crystalline bovine albumin[1] led to the conclusion that vitamin B_{12} was needed. Oleic albumin complex in medium with NH_4Cl, vitamin B_{12}, and thiamine worked. Reconstitution of the lipid extracted albumin with oleic acid was successful. Polysorbate 80 (Tween 80), a water soluble heat autoclaveable 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 bacterin producers switched to using bovine albumin polysorbate 80 medium. Great progress was made in the isolation of *L. hardjo*,[6] theretofore almost uncultivable.

1. **Schneiderman A M, Green R R, Schieller L, McClure L E & Dunn M S.** A chemically defined basal medium containing purified rabbit serum albumin. *Proc. Soc. Exp. Biol. Med.* 78:777-80. 1951. (Cited 15 times.)
2. **Marshall P B.** Measurements of aerobic respiration in *Leptospira icterohaemorrhagiae*. *J. Infec. Dis.* 84:150-2, 1949. (Cited 15 times.)
3. **Fulton J D & Spooner D F.** The metabolism of *Leptospira icterohaemorrhagiae in vitro*. *Exp. Parasitol.* 5:154-77, 1958.
4. **Helprin J J & Hiatt C W.** The effects of fatty acids on the respiration of *Leptospira icterohaemorrhagiae*. *J. Infec. Dis.* 100:136-40, 1957. (Cited 20 times.)
5. **Babudieri B & Zardi O.** Studies on the metabolism of leptospirae. I. Vitamin B_{12} as a growth factor. *Z. Vitam. Horm. Fermentforsch.* 11:299-309, 1960. (Cited 5 times.)
6. **Ellinghausen H C.** Growth culture characteristics, and antibacterial sensitivity of *Leptospira interrogans* serovar *hardjo*. *Cornell Vet.* 73:225-39, 1983.

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