

## Growth of Parasitic *Mycoplasma* Without Serum or Serum Fraction

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Received for publication 18 April 1969

Lipid-free beef heart infusion would support the growth of six strains of parasitic *Mycoplasma* when supplemented with a combination of cholesterol, lecithin, and cardiolipin.

The basal media employed in *Mycoplasma* culture are complex, generally containing beef heart infusion, peptones or protein digests, and various sera. In addition, the growth of many species is markedly stimulated by hydrolysates and extracts of yeast. Previous investigations (H. Kurzepa and P. J. VanDemark, *Bacteriol. Proc.*, p. 123, 1967) revealed that the growth stimulatory substances in yeast extract were lipids. Further investigations have identified these growth stimulatory materials as a combination of cholesterol, lecithin, and cardiolipin.

Since *Mycoplasma* medium supplemented with serum contains various levels of these lipids, investigations to quantitate *Mycoplasma* growth response to increasing levels of these nutrients required the use of a lipid-free basal medium. This lipid-free medium was prepared by the double extraction of beef heart infusion with a chloroform-methanol solvent system (2:1, v/v) by a modification of the method of Smith and Koostra (5). Extracted beef heart infusion supplemented with extracted Difco serum fraction failed to maintain the growth of several serum-requiring strains of parasitic *Mycoplasma* (Table 1). However, extracted beef heart infusion, plus yeast extract or the lipid complex of cholesterol, lecithin, or cardiolipin (suspended in absolute ethyl alcohol and added to the medium before sterilization) would support the growth of these strains in the absence of serum, serum fraction, or peptone. The latter medium will be designated HK-1 medium. In these studies, a 1% inoculum from either regular or HK-1 medium was employed, and growth was measured after a 48- to 72-hr incubation at 37 C. The levels of growth (Table 1) were determined turbidimetrically at 650 nm after the cultures were concentrated by centrifuging 10-ml volumes of culture and resuspending the pellet in 3 ml of distilled water.

Table 2 compares the growth, measured as colony counts, of strains 07, WiD, C56R, and

SW-2 when grown on Difco PPLO medium plus 1% serum fraction and 0.5% yeast extract with that obtained on HK-1 medium. Although counts are lower on the latter medium, this medium would support growth of these *Mycoplasma* strains through at least 30 serial transfers in absence of added serum or serum fraction without diminished growth. The fact that growth continued after serial transfer would rule out the possibility that these colony counts were due to a carryover of nutrients in the inoculum. These lower levels of growth probably reflect deficiencies of unknown growth-stimulatory factors in the extracted medium.

Colony diameters after one passage on HK-1 agar were significantly larger than those grown on regular PPLO medium. For example, colonies of swine strain SW-2 averaged  $128 \pm 7.6$ ,  $135 \pm 10.9$ , and  $150 \pm 22 \mu\text{m}$  in diameter after 72 hr of growth on regular PPLO medium, regular PPLO medium plus 0.5% yeast extract, and HK-1 medium, respectively.

Figure 1 illustrates the effect of variation in levels of the lipid complex on the growth of the bovine strain WiD. It is apparent from this figure that the presence and level of all three lipids is critical for optimal mycoplasmal growth.

In mycoplasmal nutrition, serum is a source of cholesterol, phospholipids, and protein (1, 3, 4). The phospholipids, acting as surface active agents, function in the aqueous solubilization of cholesterol, whereas the role of protein appears to be in detoxification and in the regulation of cholesterol uptake (4). Although the addition of yeast extract or the lipid complex to extracted beef heart infusion provides a source of cholesterol and phospholipid, no requirement for additional protein was observed in the present study. It would appear that the lipid extraction of beef heart infusion removes toxic components, e.g., long-chain fatty acids, thus alleviating the requirement of protein for detoxification. In view of the reported role of

TABLE 1. Growth<sup>a</sup> of parasitic mycoplasma on extracted media

Medium	Organism					
	<i>M. arthritidis</i> (07)	<i>M. salivarium</i>	<i>M. pneumoniae</i> (Eaton)	<i>M. pneumoniae</i> (FH)	Bovine strain WiD	Swine strain SW-2
Extracted medium <sup>b</sup>	NG <sup>c</sup>	NG	NG	NG	NG	NG
Extracted medium + 1% extracted serum fraction	NG	NG	NG	NG	NG	NG
Extracted medium + 0.5% yeast extract	0.100	0.050	0.065	0.070	0.110	0.130
Extracted medium + lipid complex <sup>d</sup>	0.115	0.110	0.090	0.070	0.135	0.100

<sup>a</sup> Optical density.

<sup>b</sup> Extracted beef heart infusion (5%).

<sup>c</sup> NG = no growth.

<sup>d</sup> Lipid complex = 0.13 ng of cardiolipin, 0.84 ng of lecithin, and 3.6 ng of cholesterol/ml of medium.

TABLE 2. Comparison of growth of parasitic *Mycoplasma* on regular PPLO and HK-1 media

Medium	Colony count (10 <sup>6</sup> colonies/ml)			
	<i>M. arthritidis</i> strain 07	Bovine strain WiD	Bovine strain C56R	Swine strain SW-2
Regular PPLO medium <sup>a</sup>	80	1.0	1.45	2.4
Regular PPLO medium + 0.5% yeast extract	89	75.0	102.0	8.8
HK-1 medium <sup>b</sup>	78	5.3	6.4	6.4

<sup>a</sup> PPLO medium (Difco) plus 1% serum fraction.

<sup>b</sup> Extracted beef heart infusion (5%) plus lipid complex (0.13 ng of cardiolipin, 0.84 ng of lecithin, and 3.6 ng of cholesterol/ml of medium).

protein in the regulation of cholesterol uptake, the failure to observe a protein requirement in the present studies is an anomaly. However, since the level of growth in extracted medium plus the lipid complex is less than that obtained with PPLO medium plus 1% serum fraction and 0.25% yeast extract, further studies may reveal that protein is among those nutrients essential for maximal growth.

Our observations on the growth of parasitic *Mycoplasma* are analogous to those of Lund and Shorb (2), who reported the culture of *M. gallisepticum* strain J in a medium without serum or protein but containing cholesterol, diacetyl tartaric acid ester of tallow monoglyceride, and enzymatic digest of casein.

This investigation was supported by Public Health Service grant AI 02658 from the National Institute of Allergy and Infectious Diseases.

We wish to express our appreciation to Carole Rehkugler and Barbara Allen for technical assistance and to P. F. Smith for the

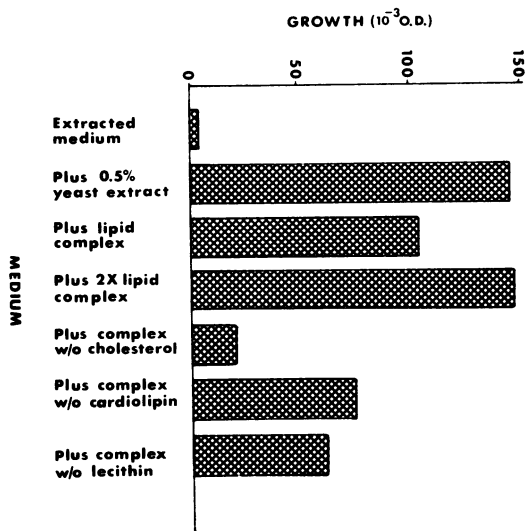


FIG. 1. Effect of variation of the lipid complex on the growth of bovine strain WiD of *Mycoplasma*. The lipid complex contained 0.13 ng of cardiolipin, 0.84 ng of lecithin, and 3.6 ng of cholesterol/ml of medium.

initial supplies of lipid-extracted beef heart infusion and serum fraction.

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