NOTES

IRREGULAR VARIATION IN CELLULAR NITROGEN ASSOCIATED WITH CULTURE AGE OF *MYCOPLASMA GALLISEPTICUM*[1]

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Methods for estimating growth of Mycoplasmataceae (pleuropneumonia-like organisms; PPLO) are often tedious (Smith, Appl. Microbiol. **4**:254, 1956), and only a few attempts have been made to correlate the data obtained by various methods. Butler and Knight (J. Gen. Microbiol. **22**:478, 1960) related colony count, turbidity, dry weight, cellular nitrogen, and nucleic acid curves for a saprophytic species (*Mycoplasma laidlawii*). Lecce and Morton (J. Bacteriol. **67**:62, 1954) related turbidity (optical density $_{420m\mu}$) to cellular nitrogen in *Mycoplasma* of human origin.

This communication presents a cellular-nitrogen growth curve for *M. gallisepticum* (an avian pathogen; Edward and Kanarek, Ann. N.Y. Acad. Sci. **79**:696, 1960) which does not correlate with optical density or dry weight, in contrast to the general case for bacteria (Oginsky and Umbreit, *Introduction to Bacterial Physiology*, 2nd ed., W. H. Freeman and Co., 1959). The curve is a function of the culture medium, and suggests ribosomal dissociation as the medium becomes limiting (Mandelstam, Bacteriol. Rev. **24**:289, 1960).

An isolate of *M. gallisepticum* (isolated by Struen Wiley, University of New Hampshire, in 1961) from a turkey with chronic respiratory disease was propagated in two media. The first medium was PPLO broth supplemented with 2% Difco PPLO serum fraction and 100 units of penicillin per ml; the second was PPLO broth enriched with 1% yeast hydrolysate and 0.5% glucose, and supplemented with 10% horse serum, 0.025% phenol red, and 500 units of penicillin per ml.

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Cultures were harvested at intervals by centrifugation (12,000 × *g* for 30 min), washed, and resuspended in one-tenth their original volume of 0.067 M phosphate buffer (pH 7.8) or distilled water. No differences in analytical values were apparent in either buffer or distilled water. Growth parameters measured were optical density at 420 mμ, dry weight, and cellular nitrogen (micro-Kjeldahl). Viable (colony) counts were performed with portions of original cultures. The data are presented in Fig. 1 and 2 on an original culture basis. Nucleic acid curves of the isolate grown in enriched PPLO broth were subsequently studied by the methods of Lynn and Smith (J. Bacteriol. **74**:811, 1957) to evaluate the unusual cellular-nitrogen curve (Fig. 3).

Approximately tenfold increases in viable cells were found in both media. In PPLO broth there is good correlation between optical density, dry weight, and cellular nitrogen (Fig. 1). Enriched PPLO broth (Fig. 2) promotes growth until some factor, possibly an amino acid, becomes limiting. Cells then lose viability logarithmically, but apparently remain intact as evidenced by the increasing optical density and dry weight. However, nucleic acids and nitrogen are lost rapidly, which suggests ribosomal dissociation without lysis followed by subsequent breakdown of protein as is the general case for microorganisms with various deficiencies (Mandelstam). The remaining viable cells appear to reincorporate the limiting factor(s) and undergo a brief period of increased ribonucleic acid and nitrogen incorporation. In previous studies on *M. gallisepticum*, Gill (J. Bacteriol. **83**:213, 1962) reported the release of peptides to the nutrient which subsequently disappeared as the culture aged. There was no detectable increase in viable cells at 30 to 42 hr as would be expected if appreciably more

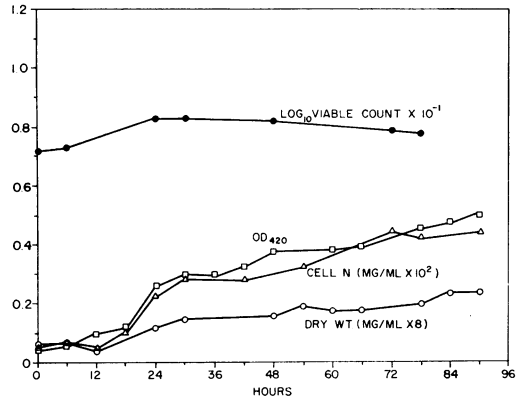

FIG. 1. *Growth curves of Mycoplasma gallisepticum in PPLO broth.*

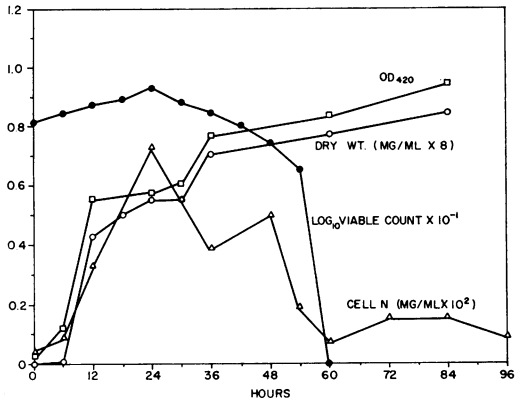

FIG. 2. *Growth curves of Mycoplasma gallisepticum in enriched PPLO broth.*

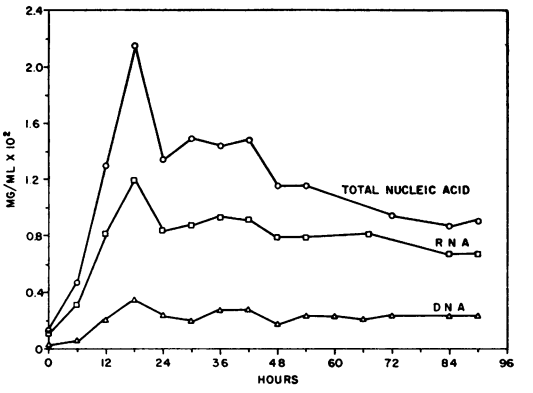

FIG. 3. *Nucleic acids of Mycoplasma gallisepticum in enriched PPLO broth.*

deoxyribonucleic acid were found. The increases in optical density and dry weight were similar to those reported by Toennies (Abstr., 142nd Am. Chem. Soc. Meeting, 1962) for particular amino acid deficiencies in *Streptococcus faecalis*. The curves in Fig. 2 are corrected for horse serum proteins precipitated by acid formed during the fermentation of glucose.

A similar double-peak curve of steroid content vs. culture age in a human PPLO was observed by Lynn and Smith (Ann. N.Y. Acad. Sci. **79**:493, 1960).

It is evident that optical density does not necessarily correlate with cell nitrogen for the Mycoplasmataceae. Nutritional factors may alter this relationship, which must be verified for each organism in the desired medium.

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IMMUNOCHEMICAL DIAGNOSIS OF THE LINKAGE OF D-MANNOSE RESIDUES

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On the basis of differential quantitative microanalyses of the cross-precipitation of galactomannans and of yeast mannan in antityphoid and antiparatyphoid B sera, Heidelberger and Cordoba (J. Exptl. Med. **104**:375, 1956) concluded that the linkages of the mannose residues in the O antigen of *Salmonella typhi* would be found to be mainly 1,2-, or 1,3-, or both. That this view was correct was indicated by the results of oxidation of the typhoid polysaccharide with periodate