

The Preparation of Membranes of Some Human T-Mycoplasmas and the Analysis of Their Carbohydrate Content (38965)

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Extensive chemical analyses have been made of the membranes of the classical mycoplasmas, especially *Acholeplasma laidlawii* (1, 2). In contrast only suspensions of whole cells of the T-mycoplasmas have been examined for their constituent proteins by polyacrylamide gel electrophoresis (3). Purified cell membranes of four laboratory strains of T-mycoplasmas and three isolates from patients with nonspecific urethritis were prepared and analysed for their carbohydrate content. The results are reported below.

Materials and Methods. *T-mycoplasma T-McA*, *T-960*, *T-27*, and *T-58* are laboratory strains while *T-210*, *T-213*, and *T-220* are isolates from patients with nonspecific urethritis. The composition of the T-broth pH 6.5 and T-agar pH 6.5 (4), and the techniques for making colony counts of viable T-mycoplasmas (4) and for preparing single-cell suspensions by sonic treatment on a Raytheon ultrasonic oscillator DF 101 cooled with ice water (5) have been reported. T-mycoplasma T-broth cultures in screw-capped bottles containing 500 ml medium were incubated in a water bath at $37^{\circ} \pm 0.50^{\circ}$ until the T-mycoplasmas reached the late exponential phase when the broth contained about 1×10^6 T-mycoplasmas/ml. T-agar plates were incubated in a 37° humidified incubator gassed with 5-10% CO₂ in air.

Electron microscopy of negative stained preparations. A drop of the membrane suspension was placed on a Formvar coated copper grid and any excess removed by blotting the edge of the grid. The membranes were negatively stained by putting on the grid a loopful of 1% molybdic acid adjusted to pH 6.8-7.0 with ammonium hydroxide.

The preparations were examined by a Hitachi HU 12 electron microscope with an accelerating voltage of 75 kV.

Carbohydrate (neutral sugars) analysis. Between 1.0 and 2.5 mg of dried T-mycoplasma membrane was hydrolysed in a sealed vial by 2 N HCl at 100° for 8 hr, the hydrolysate dried at room temperature over NaOH pellets and resuspended in 1 ml distilled water. Quantitative and qualitative determinations of the neutral sugars were obtained by Kesler's method (6) using a Technicon Autoanalyser.

Results. The technique for obtaining T-mycoplasma membranes was elucidated using *T-mycoplasma T-McA*. In the expectation that the T-mycoplasmas would be as susceptible to osmotic lysis as *A. laidlawii* (1, 2), an aqueous suspension was prepared by centrifuging 100 ml of a T-broth culture of *T-McA* at 14,500g for 30 min (7), decanting the medium, and resuspending the pellet in distilled water. Then attempts were made to lyse the T-mycoplasmas first by sonication for 15 min at full power in a Raytheon ultrasonic oscillator DF 101 cooled with iced water, secondly by rapid freezing and slow thawing at room temperature, and thirdly by passing the aqueous suspension three times through an Aminco French Press at 18,000 psi (American Instrument Co., Silver Spring, Md.). After each manipulation the suspension was centrifuged at 14,500g for 30 min to deposit the T-mycoplasmas followed by centrifugation of the supernatant at 37,500g for 60 min to sediment the membranes (1). The T-mycoplasmas were examined by interference microscopy (7) and the negatively stained membranes by EM. Few T-mycoplasmas were lysed by these techniques. Therefore an aqueous suspension was prepared and sonicated to disperse any protein precipitates

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and disrupt any aggregates of T-mycoplasmas into single cells which were assayed by viable counts. Thereafter, the suspension was passed four times through a Sorvall cell fractionator at 40,000 psi and again assayed for viable mycoplasmas. Approximately 20% of the T-mycoplasmas remained viable. Therefore, the T-mycoplasmas were separated from the membranes by centrifugation twice at 14,500g for 30 min to deposit the cells, followed by centrifugation of the supernatant at 37,500g for 60 min to deposit the membranes. An aqueous suspension of the membranes did not contain any viable cells and only membrane ghosts resembling collapsed sacks were seen by EM (Fig. 1).

Therefore membrane preparations were prepared routinely as follows: Six bottles containing 500 ml exponential phase T-broth cultures were centrifuged at 14,500g for 30 min and the supernatant decanted. The pellets of T-mycoplasmas were resuspended in phosphate buffered saline (PBS), pooled, sonicated for 1 min, washed twice in PBS to eliminate any T-broth constituents, and resuspended in 50 ml distilled water. The aqueous suspension was passed four times through the Sorvall cell fractionator at 40,000 psi, and centrifuged twice at 14,500g for 30 min to sediment any whole T-mycoplasmas. Then the supernatant was centrifuged at 37,500g for 60 min to deposit the

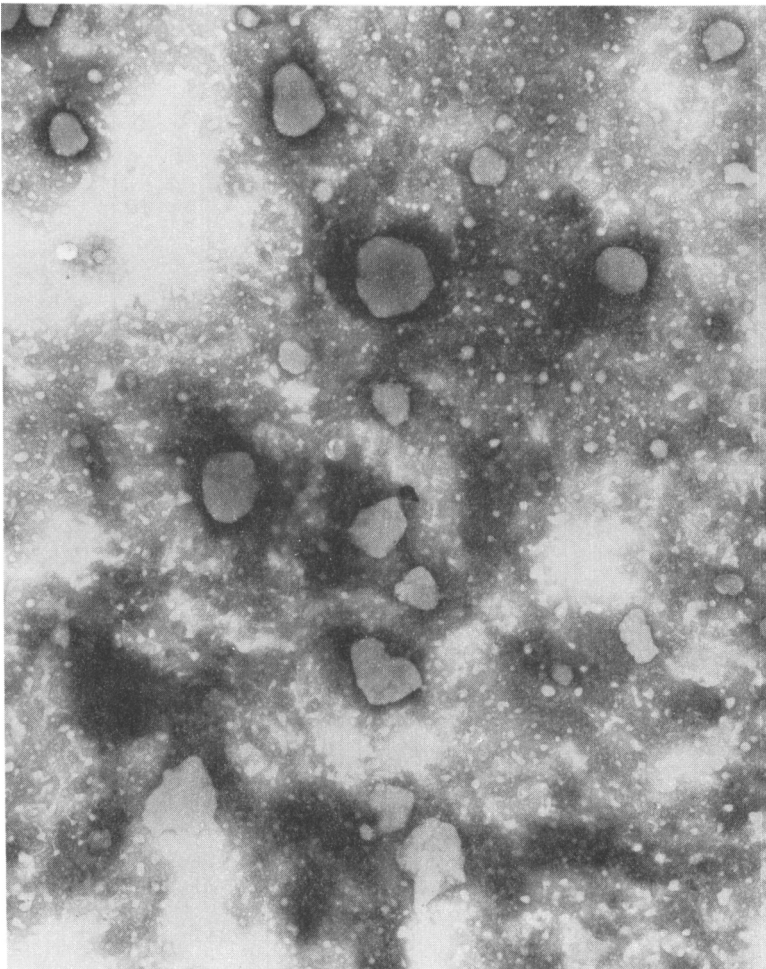


FIG. 1. Electron micrograph of a purified T-mycoplasma membrane preparation negatively stained with 1% molybdic acid. The membrane ghosts only partially stain and resemble collapsed sacks ($\times 16,000$).

TABLE I. CARBOHYDRATE CONTENT OF MEMBRANE PREPARATIONS OF SEVEN T-MYCOPLASMAS

T-Mycoplasma	Mannose	% Neutral sugar ^a Galactose	Glucose
T-McA	0.57	0.21	1.64
T-213	0.20	0.20	3.32
T-210	0.23	0.28	3.83
T-220	0.21	0.25	3.93
T-960	0.26	0.1	4.10
T-58	0.24	0.15	5.10
T-27	0.20	0.20	5.45

^a Total neutral sugar expressed as a percentage of the dry weight of the membrane.

membranes which were resuspended in a minimal volume of distilled water and checked for purity by EM. Thereafter the membranes were lyophilised for chemical analysis.

Carbohydrate (neutral sugar) analysis. Membrane preparations were made from duplicate cultures of the seven T-mycoplasmas and each was analysed separately. The membranes contained three neutral sugars, mannose, galactose and glucose (Table I). The mannose content of six strains was within the range 0.20–0.25% while the other strain, *T-McA*, contained 0.57%. The galactose content of six strains was between 0.15 and 0.28% while strain *T-960* contained less than 0.1% which was too low to estimate accurately from the chromatograms. The other estimations were within the standard error of the techniques. The laboratory strain *T-McA* contained only 1.64% glucose while the other laboratory strains *T-960*, *T-58*, and *T-27* had, respectively, 4.10, 5.10, and 5.45%. The three isolates from non-specific urethritis patients, *T-213*, *T-210*, and *T-220* had membranes containing, respectively, 3.32, 3.83, and 3.93% glucose.

Discussion. These seven T-mycoplasmas were studied because biological tests indicate that they are different species (Furness, unpublished). Moreover, some do not have the same surface antigens (8) which is indicative that their membranes differ. The neutral sugar analysis of the T-mycoplasma mem-

branes suggested that the carbohydrates in the membranes of both the T-mycoplasmas and classical mycoplasmas (1) are similar. They contain mannose, galactose and glucose ranging from about 1 to 7% dry wt with glucose usually being the primary neutral sugar. However, the most important observation was the great resistance of the T-mycoplasmas to physical stresses as compared to the classical mycoplasmas (1, 2). This probably is due to differences in some of the other components of the T-mycoplasma membrane which now can be analysed.

Summary. Purified membranes were prepared from seven human T-mycoplasmas of which four are laboratory strains and three isolates from patients with nonspecific urethritis. The T-mycoplasmas were resistant to osmotic shock and sonication. Membranes were obtained only after lysing most of the T-mycoplasmas by four passes through a cell fractionator at 40,000 psi and separating the membranes from the unlysed cells by differential centrifugation. The membranes contained between 1 and 7% carbohydrates by dry weight. Mannose, galactose, and glucose were identified with glucose in the largest concentration.

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1. Razin, S., Argaman, M., and Avigan, J., *J. Gen. Microbiol.* **33**, 447 (1963).
2. Razin, S., *J. Gen. Microbiol.* **33**, 471 (1964).
3. Taylor-Robinson, D. and Martin-Bourgon, C., Watanabe, T., and Addey, J. P., *J. Gen. Microbiol.* **68**, 97 (1971).
4. Furness, G., *J. Infect. Dis.* **127**, 9 (1973).
5. Furness, G., *Appl. Microbiol.* **18**, 360 (1969).
6. Kesler, R. B., *Anal. Chem.* **39**, 1416 (1967).
7. Whitescarver, J. and Furness, G., *J. Med. Microbiol.*, in press.
8. Black, F. T., Birch-Andersen, A. and Freundt, E. A., *J. Cell Biol.* **111**, 254 (1972).

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