20. Wilcoxon's nonparametric matched-pairs, signed-ranks test was used for statistical comparison of the 12 all-day sample pairs resulting; this test avoided the difficulty of variation due to differences in flight intensity on different days.

3,3-MCH was synthesized by ChemSampCo, Columbus, Ohio. GC/MS indicated 95% purity and 0.6% of the active 3,2-MCH isomer present. The 3,3-MCH was held at −15 °C until needed, and replaced in the field weekly to prevent conversion into enough of the more stable 3,2-MCH to interfere with the tests. After 1 year, the purity of 3,3-MCH held at −15 °C was unchanged according to GC/MS. Frontalin 98.5% was also from ChemSampCo, and α-pinene 95% from K & K Laboratories, Plainview, N.Y.

*Results and discussion.* Response of flying *D. pseudotsugae* to attractant in sticky traps was about 77% less than response to the control traps, a highly significant difference. However, the sections of windthrown trees with 3,3-MCH placed along them did not have significantly fewer attacks than the untreated portions of the trees (table). These results are in marked contrast to previous tests of 3,2-MCH using the same delivery system. Addition of 3,2-MCH to sticky traps baited with frontalin and α-pinene near Hood River, Oregon, decreased the beetle catch by 96%[3]. Also, 3,2-MCH reduced by 96%[4] the number of attacks on felled trees in 3 locations in Oregon and Idaho.

Response of flying *Dendroctonus pseudotsugae* to field test of 3,3-MCH during spring flight: A, attacks per 0.5 m² bark sample on windthrown trees; B, beetles collected on sticky traps to attractant with 3,3-MCH and without (control)

| Test | Control $\bar{x} \pm 95\%$ CI | ♂/♀ | With 3,3-MCH $\bar{x} \pm 95\%$ CI | ♂/♀ | n | Significance |
|---|---|---|---|---|---|---|
| A Trees | $6.9 \pm 1.65$ | - | $4.1 \pm 1.05$ | - | 24 | NS $p < 0.05$ |
| B Traps | $9.1 \pm 3.03$ | 4.4 | $2.1 \pm 2.08$ | 3.8 | 12 | ** $p < 0.01$ |

Preparation and testing of 3,3-MCH is difficult because it begins to convert into its more stable 3,2-isomer at temperatures favorable for beetle flight. Our field bioassay of 3,3-MCH was begun in 1976, but the results suggesting that it had an inhibitive effect on flight aggregation were invalidated when a later GC/MS of our stock compound revealed a 5–8% conversion to the active 3,2-MCH isomer[7]. Since our field tests have shown 3,3-MCH to have only a slight effect on flight aggregation, at about the same level as several MCH analogues tested[8], its biological function remains obscure. Since it is less stable than its isomer, it is possible that 3,3-MCH is a precursor to 3,2-MCH, or it may have more subtle primer or releaser effects during male premating behavior in the gallery, as does 3,2-MCH[9].

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2 J.A. Rudinsky, M. Morgan, L.M. Libbey and R.R. Michael, J. envir. Ent. *2*, 505 (1973).

3 J.A. Rudinsky, M.M. Furniss, L.N. Kline and R.F. Schmitz, Can. J. Ent. *104*, 815 (1972).

4 M.M. Furniss, G.E. Daterman, L.N. Kline, M.D. McGregor, G.C. Trostle, L.F. Pettinger and J.A. Rudinsky, Can. J. Ent. *106*, 381 (1974).

5 L.M. Libbey, M.E. Morgan, T.B. Putnam and J.A. Rudinsky, J. Insect Physiol. *22*, 871 (1976).

6 M.M. Furniss, R.F. Schmitz and J.A. Rudinsky, Ann. ent. Soc. Am. *65*, 1227 (1972).

7 J.A. Rudinsky and L.C. Ryker, in: Comportement des Insectes et Milieu Trophique, p. 195. Ed. V. Labeyrie. Coll. du C.N.R.S. No. 265, Tours, France, 1977.

8 J.A. Rudinsky, L.N. Kline and J.D. Diekman, J. econ. Ent. *68*, 527 (1975).

9 J.A. Rudinsky and L.C. Ryker, J. Insect Physiol. *22*, 997 (1976).

## Microorganisms seen by scanning and transmission electron microscopy in Legionnaires' disease from human lung[1]

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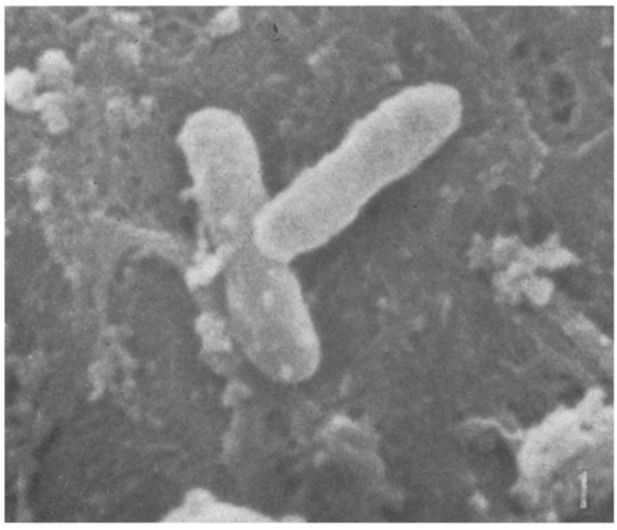
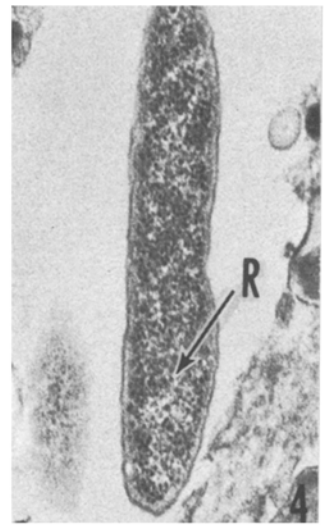
*Summary.* In addition to several anomalous structures, other general forms of definitely rod-shaped microorganisms have been found by scanning and transmission electron microscopy in the lung tissue taken at autopsy from a patient who succumbed to confirmed Legionnaires' disease with extensive necrotizing lobar pneumonia. The microorganisms were greatly varied in size and shape. They were micrographed in the act of fission. These forms have been found to some extent throughout the tissue. No nickel was demonstrated, either in the lung tissue or in the microorganisms.

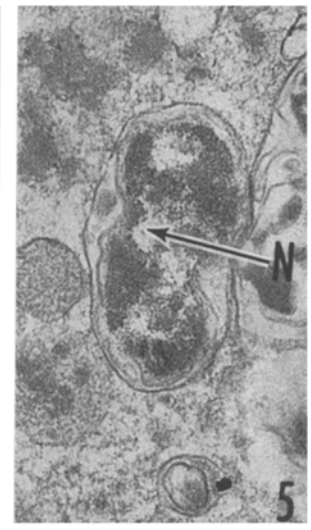
An epidemic of acute febrile respiratory disease broke out among persons attending the American Legion convention in Philadelphia in 1976, and earned for it the name 'Legionnaires' disease'. Although an organism has since been isolated and cultured from the lungs of patients with the disease[2,3], only 2 studies of it directly in tissue sections have been reported by transmission electron microscopy (TEM)[4,5] and none by scanning electron microscopy (SEM). In the present report both SEM and TEM are being used to study the causative organism more thoroughly, and preliminary results are described.

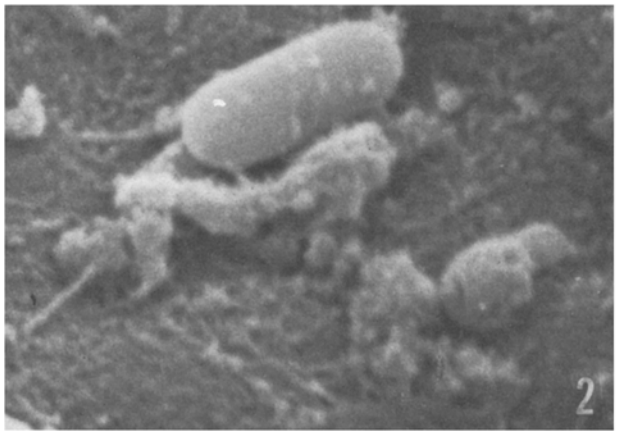
The lung tissue was taken at autopsy from a patient who died in June 1977 with extensive necrotizing lobar pneumonia. The diagnosis of Legionnaires' disease was made by specific fluorescent antibody and silver impregnation staining[3] and was confirmed by the Cummunicable Disease Center in Atlanta, Georgia, USA.

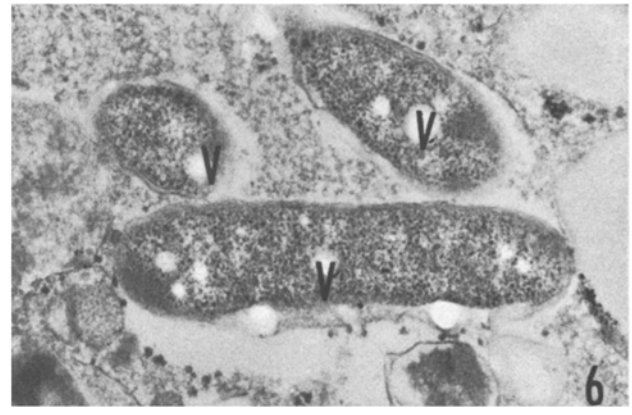
*Materials and methods.* The specimens of formalin-fixed autopsy material were washed in veronal acetate buffer. A part was separated for TEM and the remainder processed for SEM. For SEM the pieces were critical-point dried in $CO_2$ and sputter-coated with gold-palladium after dehydration in graded ethanols, including 3 changes of absolute alcohol. Micrographs were taken at 20 kV in an ETEC Autoscan. For TEM other pieces were post-fixed in phosphate buffered or veronal acetate buffered osmic acid,

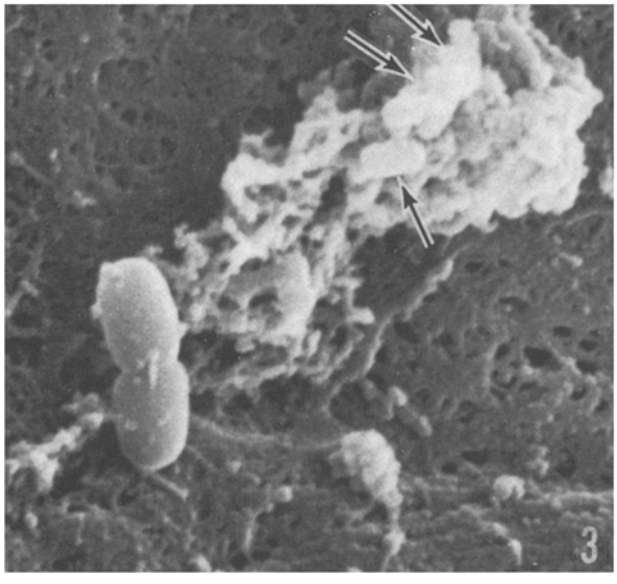


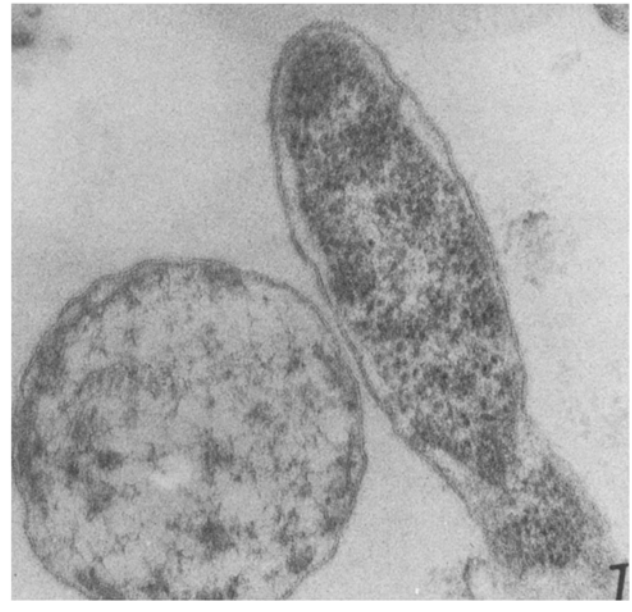




Figures 1–3. SEM of microorganisms in human lung from Legionnaires' disease at autopsy. Fig. 1. Rod-shaped bodies, relatively long and thin. × 25,000. Fig. 2. Sausage-shaped bodies, shorter and plumper. × 25,000. Fig. 3. Microorganism in fission. × 20,000.

Figures 4–7. TEM of microorganisms in human lung from Legionnaires' disease at autopsy. Rod-shaped forms in long transversalis and oblique section showing nuclear material (N), vacuoles (V) and ribosomes (R). Figs. 4 and 5. × 30,000. Fig. 6. × 35,000. Fig. 7. × 45,000.

before dehydration in graded ethanols through propylene oxide into epon 812 or araldite. Some of the pieces were stained in block with uranyl acetate in pH 5 veronal acetate buffer. Sections were cut approximately 200-nm-thick and contrasted with uranyl acetate and lead citrate for examination.

*Results. Scanning electron microscopy.* The necrosis in the lung tissue was so advanced that it was only possible at low magnifications to identify the tissue at all as lung, and then only by recognition of a few remaining alveolar spaces and their interalveolar septa. Even these sparse alveoli were badly occluded with cells and debris. In them no type II epithelial cells were found but there were type I cells and a pletheora of other loose cells, many of which resembled macrophages but which could as well be polymorphonuclear leukocytes and lymphocytes active in the necrosis.

Small microorganisms were found extracellularly, usually lying on the free surfaces of the few recognizable alveoli, particularly in the more badly damaged regions of the tissue. They varied considerably in their size, shape and morphological appearance by SEM (figures 1–3). They were often found in the process of binary fission (figure 3). Some (figure 1) were relatively long and thin, with slightly tapered, sometimes almost pointed tips and uneven outlines. They were generally describable as 'rod-like' and frequently bore small surface bumps (diameters 0.06 μm), which we have reason to believe are not contaminant. Their surfaces were uneven and often bore low ridges or folds. Others, describable as 'sausage-shaped' (figure 2), were measurably shorter and plumper than the first, with well rounded sides and tips and smoother surfaces. These, too, had small bumps on their surfaces but no folds or ridges. If they are assumed to be the same organism as the first, the mean length and width for the combined total distribution of 71 of them were 1.84 and 0.56 μm, respectively, with a ratio of length to width of 3.3, in close agreement with measurements reported by others[5] from TEM of the microorganism.

*Transmission electron microscopy.* In thin sections (figures 4–6), the microorganisms possess a limiting cell envelope comprised of a triple membrane, 2 dark membranes separated by a clear space. The thickness of the triple membrane is 11–14 nm. The outer membrane was sometimes loose and undulating, in agreement with the SEM observations of surface folds or ridges. The cell contents are granular due to the presence of small particles (25 nm), the same size as ribosomes (figures 4–6). Fine thread-like structures (diameter 6 nm), were sometimes seen in their cytoplasm (figures 4 and 5), and many, but not all of the sectioned organisms demonstrated vacuoles within them, ranging from 30 to 160 nm in diameter (figure 6). Nuclear material was seen in some (figure 5), but not in all of them.

*Discussion.* The morphological characteristics of the microorganisms reported here from both SEM and TEM agree closely with those reported from TEM alone by others[5] for the responsible microorganism, described as the etiological factor in Legionnaires' disease. In addition, many other forms as well as a wider distribution of possible rod bodies have been sighted by SEM. A full discussion and description of these forms will be the subject of a later report. For example, in addition to the forms of microorganisms reported here, there is a great variety of rounded or oval bodies with a wide distribution of diameters. Some of these are very small (length and/or width approximately 0.1–0.3 μm) and representatives can be seen at the arrows in figure 3. Others can be quite large, as in figure 7, where the triple-membraned, rounded body is over 3 μm in diameter. These rounded bodies or spheroplasts resemble mycoplasmas[6] by TEM in thin section. However, in sections they do not seem to contain the small, ribosome-like particles which were characteristic of the bacterium-or rickettsia-like organism in thin section.

Efforts to detect the presence of the element nickel in the tissue and bacterium by both fluorescent X-ray and energy dispersive X-ray analysis were unrewarding and it was concluded that no nickel was present. This effort was stimulated by the suggestion put forth by Dr W. Sunderman, Jr at the time of the initial outbreak in Philadelphia. He claimed that nickel was present in the tissues of patients who died of Legionnaires disease.

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2 J.E. McDade, C.C. Shepard, D.W. Fraser, T.R. Tsai, M.A. Redus and W.R. Dowdle, N. Engl. J. Med. *297*, 1197 (1977).
3 F.W. Chandler, M.D. Hicklin and J.A. Blackmon, N. Engl. J. Med. *297*, 1218 (1977).
4 H.J. White, C.N. Sun and A. Hui, Human Path. *10*, 96 (1979).
5 F.G. Rodgers, A.D. Macrae and M.J. Lewis, Nature *272*, 825 (1978).
6 D.R. Anderson, in: Mycoplasmatales and the L-Phase of Bacteria, p.365. Ed. L. Hayflick. Appleton-Century-Crofts, Meredith Corp., New York 1969.

## Reassociation of eukaryotic ribosomal subunits by a factor from rat ascites hepatoma cytosol

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*Summary.* Post-ribosomal supernatant extracts from Yoshida AH 130 ascites hepatoma cells promote the in vitro association of ribosomal subunits at low $Mg^{2+}$ concentration. Comparable extracts from rat liver show, on the contrary, dissociation factor activity on ribosome monomers.

The dissociation of ribosome monomers into subparticles is a requisite for the initiation of protein synthesis[2]. Factors with that activity have been found in the cytosol and in the extract prepared by high salt wash from ribosomes in a number of eukaryotic cell types[3–8], including the mouse ascites cells[9] and the rat AH 130 Yoshida ascites hepatoma[10].

The in vitro activity of initiation factors appears to increase in transplantable hepatomas[11,12]. However, the in vivo sedimentation patterns of ribosomes obtained from these