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Authors' reply

It was interesting to read the evaluation of our paper (1) by Professor J. Maniloff, the pioneer of ultrastructural studies of *M. gallisepticum* (2,3).

In his letter above, J. Maniloff points out the general agreement in the parameters of the tubular structure measured by EM and optical diffraction, reported by him previously (2) and by us in the paper discussed (1). Although the optical transforms published in the two papers (2,1) are undoubtedly those of a helix, we cannot agree with his assertion, particularly regarding the quantity of particles (subunits) per turn. Values of 3.6 and 3.9 in our paper (1) do not correspond to the quantity of subunits per turn of the spiral. Meridional reflections (M) shown in Fig. 6(b) of our paper (1), considered by J. Maniloff as the 5th layer line, are actually the single meridional layer line reflecting the spiral pitch. Four layer lines on the right-hand side of the same Fig. 6(b) belong to another set of reflections, which is demonstrated by a non-integer (3.6 or 3.9) P/p (pitch/period) ratio, calculated from the two different sets of reflections. This is the reason why the results of our diffraction pattern

analysis do not allow us to predict the subunit structure of the helix and, more importantly do not permit the construction of models, based on ribosomal subunits or subunits of any other nature.

With regard to the electron-dense particles revealed by EM in our work (1) and shown previously by Maniloff (3), interpretation of the results is also different. We see 4–5 electron-dense units in cross-sections of the tubules. Using 5 nm colloidal gold particles (Sigma) as marker, we have determined the diameter of these particles to be 7 nm, certainly no more than 10 nm, which is different from Maniloff's estimation (17 nm) and does not correspond to the diameter of the 70S ribosomal particle.

Protein with a molecular mass of 40 kDa, reacting with antibodies to eukaryotic tubulin was found both on immunoblots and directly in the content of the tubular structures (1). Initially our working hypothesis was that some cytoskeletal spiral thread might be 'decorated' by ribosomes. But results of our image analysis by optical diffraction does not correspond to any ribosomal model.

The intracellular spatial distribution of the tubular structures of *M. gallisepticum* is highly organized. Loop-shaped tubules are arranged regularly along the cell membrane (1). In our EM experiments with *M. gallisepticum* cells, using standard chemical fixation or freeze-

substitution techniques before or after harvesting by centrifugation, tubular structures were visualized in all cases over several years. It is difficult to accept that the complex systems of tubular structures revealed is a result of a preparative artifact.

Our ultrastructural and immunological data indicate that tubulin-like proteins form part of the tubular structures of *M. gallisepticum*. However, we do not have enough data at the moment to propose any new subunit model for the tubular structures. The system of tubules revealed in *M. gallisepticum* is possibly involved in motility (1).

E. Korolev, Ya. Komissarchik and S. Borchsenius*

Institute of Cytology, Russian Academy of Science, Tikhoretsky Avenue, 4, 194064, St Petersburg, Russia.

*For correspondence. Tel: +7 812 2471829.

Fax: +7 812 2470341.

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