than in a salt solution, and the enhancement of the relaxation rates is much larger than that for the extracellular sodium ions. The NMR-invisible fraction of the cesium and the rubidium signal must have a relaxation rate R_{2f} larger than 20,000 sec^{-1}, which is the detection limit of our spectrometer. The relaxation decay of $^{39}K^+$ ions in another Halobacterium sp. has been observed[19]. In that case a considerable enhancement of the relaxation rate seems to exist as well. The large enhancement of R_{2s} and a large R_{2f} both indicate that slow fluctuations in the molecular environment of the intracellular ions, at rates slower than about 10^8 sec^{-1}, contribute substantially to the transverse NMR relaxation.

We can contrast this with the rate of fluctuation of the hydration sphere of an alkali ion in solution, which is of the order of 10^{11} sec^{-1}[20]. Slow changes in the environment of the intracellular ions are expected if the ions are bound. The NMR results therefore substantiate that the intracellular ions K^+, Rb^+ and Cs^+ are somehow bound in Halobacterium.

The meaning of the 2 resolved fractions in the relaxation of the cesium signal is not clear. One might speculate that they reflect 2 different types of intracellular environment, each characterized by different values of their relaxation rate R_{2s}. This possibility is not entirely hypothetical, as other experiments on intracellular K^+ point to 2 different types of ionic environments for the intracellular ions[8].

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Contact inhibition and the movement of metal, glass and plastic beads within solid tissues

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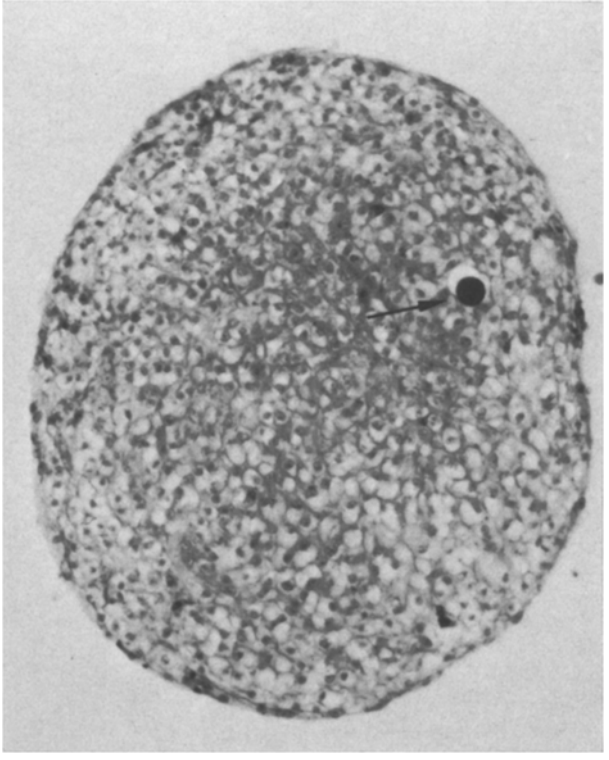
Summary. Cell-size spheres of metal, glass and plastic can move from surface to subsurface positions within solid tissue masses in culture, demonstrating that the observed movement of cells in similar circumstances may not be due to 'active cell locomotion'.

If 'contact inhibition of cell movement' stops cell locomotion in the direction of contact with another cell, then cells in contact on all sides with other cells might be expected to show little or no movement[1,2]. Evidence for just such an expectation has been reported[3,4]. A small piece of embryonic chick tissue was allowed to fuse in culture with another bit of embryonic tissue previously labeled with tritiated thymidine. Autoradiographic inspection of histological sections prepared after several days of culture revealed essentially no movement of labeled cells across the boundary between the labeled and the unlabeled tissues.



Section through a fragment of heart tissue. Arrow indicates a stainless steel sphere which was seeded onto the surface of the fragment and has moved to the interior during 3 days of culture (×250).

Apparently contradictory results have been reported with a slightly different experimental procedure[4,5]. In this experiment single, labeled cells were seeded onto the surfaces of unlabeled tissue masses in culture and the subsequent movement of labeled cells was monitored by autoradiography. After 2–3 days of culture a substantial number of single, labeled cells were found within the interiors of tissue masses. Control experiments demonstrated that the observed cellular displacement was due neither to the presence of holes or fissures in the tissue masses through which single cells passively circulated, nor to the pre-treatment of isolated cells with proteolytic enzymes. The same tissues were used in these seeding experiments as were used in the earlier fusion experiments.

Evidence is reported in this communication which indicates clearly that the displacement of single cells from the surface to the interior of a tissue mass is not alone sufficient evidence to conclude that cells 'actively move'[6] in such cases. I repeated the seeding experiments substituting beads of metal, glass and plastic for labeled cells. The beads (Duke Scientific Corporation, Palo Alto, California) were spherical and of approximately cell size: polystyrene, 9.8 micron average diameter; aluminum, 7–15 microns; glass, 10–15 microns; and stainless steel, 8–18 microns. Smooth, round fragments of 5 day em-

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bryonic chick heart or liver tissue were cultured in hanging drops[7] (minimum essential medium plus 10% horse serum and 50 mcg/ml gentamicin) at 37 °C in a water-saturated atmosphere containing 5% CO_2 together with a suspension of beads. 15–20 droplet cultures were maintained for each combination of tissue and bead type, with one tissue sphere per droplet and several hundred to several million beads per ml. Tissue masses with adhering beads were fixed after several hours and after several days, embedded and sectioned at 10 microns and stained. After 2–6 h of culture beads were confined to the surfaces of the tissue masses. However, tissue fixed after 3–4 days of culture showed, in addition to many surface beads, a few individual beads within the interiors of the tissues. In some cases single beads were found many cell diameters beneath the tissue surface (figure).

These observations are interesting for at least 2 reasons. First, the interpretation that individual cells are 'actively motile' within solidly packed cell masses because they are found to 'move' from the surface to the interior is not the only possible explanation. It is obvious that beads which move from sufrace to interior positions do not do so by active locomotion. Although these results do not rule out the possibility of individual cell movement, neither are they inconsistent with other possibilities (e.g., active 'engulfment' of individual non-motile cells by solid tissue masses or the continual zipping together and unzipping of cell membranes as weaker cell-cell adhesions are passively exchanged for stronger ones).

The second reason these observations are of interest is that they may offer an experimental system for investigating putative cell surface moieties involved in cell adhesion and cell movement. It is possible to link molecules, specific enzymes or antibodies for instance, to carboxylated polystyrene beads similar to the ones used here[8]. Beads could be prepared in conjugation with various particular molecules and the movement or non-movement of individual beads within solid tissues could be correlated with the presence or absence of these molecules. Hopefully, this might aid in our understanding of potentially interesting cell surface molecules which may be implicated in such real single cell movements in solid tissues as primordial germ cell and neural crest cell migrations during vertebrate embryogenesis, and malignant cell invasiveness.

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Occurrence of β-chitin in the cuticle of a Pentastomid Raillietiella gowrii[1]

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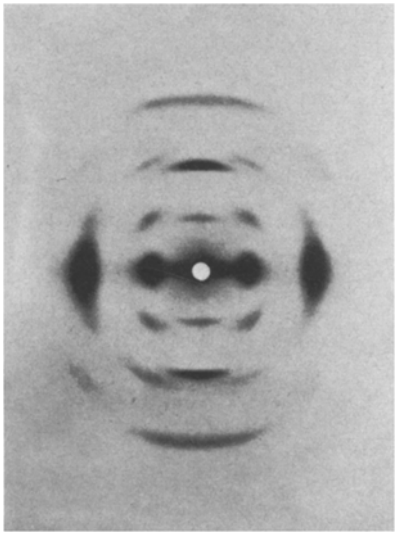
Summary. The purified chitin from the cuticle of a pentastomid was examined by X-ray method. The X-ray photograph discloses that the chitin in question is of β-type. Since the arthropod cuticle contains α-chitin, it is suggested that Pentastomida may be considered an independent phylum.

The Pentastomida are an aberrent group of parasites whose phylogenetic position is controversial. Heymons[2] and Kaestner[3] observe that the appearance of the coelomic pouches, distinct neuromeres and appendage primordia, the longitudinal anastomosis between lateral nerves to the sense organs and the parietal musculature are the annelidan characters found in Pentastomida. But Shipley[4] and Storer and Usinger[5] note that the Pentastomida show affinities to arthropods in having a chitinous cuticle, striated muscles and a segmentally organized nerve cord. The 2 pairs of legs in Pentastomida are like those of Tardigrada and Onychophora. A third view is held by observers like Trainer et al.[6] who find that the structure of the body wall and the cuticle in Pentastomida are simpler than that of either the Onychophora or Tardigrada and suggest giving the group the status of an independent phylum.



X-ray photograph of purified chitin from the cuticle of Raillietiella gowrii, taken after drying the material in vacuo.

It may be specially noted in this context that the presence of chitin in the cuticle has long been used as a strong point in the argument for the affinities of the pentastomes to Arthropoda. But it is known that Annelida, Coelenterata, Mollusca and Brachiopoda also have chitin in their cuticles as have Arthropoda. In the former groups, the chitin is of β-form, while in the latter the same

1 Thanks are due to Prof. Dr G. Sundara Rajulu for guidance and encouragement.

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