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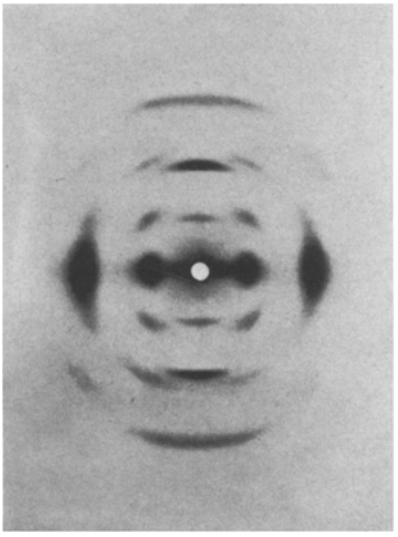
Summary. The purified chitin from the cuticle of a pentastomid was examined by X-ray method. The X-ray photograph discloses that the chitin in question is of β-type. Since the arthropod cuticle contains α-chitin, it is suggested that Pentastomida may be considered an independent phylum.

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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is of α-type[7]. It is therefore thought that X-ray characterization of the pentastomid chitin may throw some light on the problem.

The material used is the broader sheets of cuticle of the adults of Raillietiella gowrii of size 9.2 cm long and 3.3 mm thick. The chitin of the cuticle was purified by boiling the material in 5% KoH solution for 24 h, followed by prolonged washing in cold water and the purified material was used for X-ray studies. The X-ray photograph was taken in a cylindrical camera after drying the material in vacuo over phosphorous pentoxide. The accompanying figure is the X-ray photograph of the cuticle of Raillietiella gowrii. The principal axial spacings are meridian: 10.1, 5.1 and 2.55 and equatorial: 23.5, 10.34 and 4.4. These are features said to be associated with β-chitin[7,8].

These results may suggest that though the cuticle in Pentastomida contains chitin, it is of β-type, unlike in Arthropoda where the chitin is of α-type. In view of these observations, it is suggested that Pentastomida may best be kept in an independent phylum.

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Changes in acid phosphatase activity in 6 tissues of the fifth instar milkweed bug, Oncopeltus fasciatus[1]

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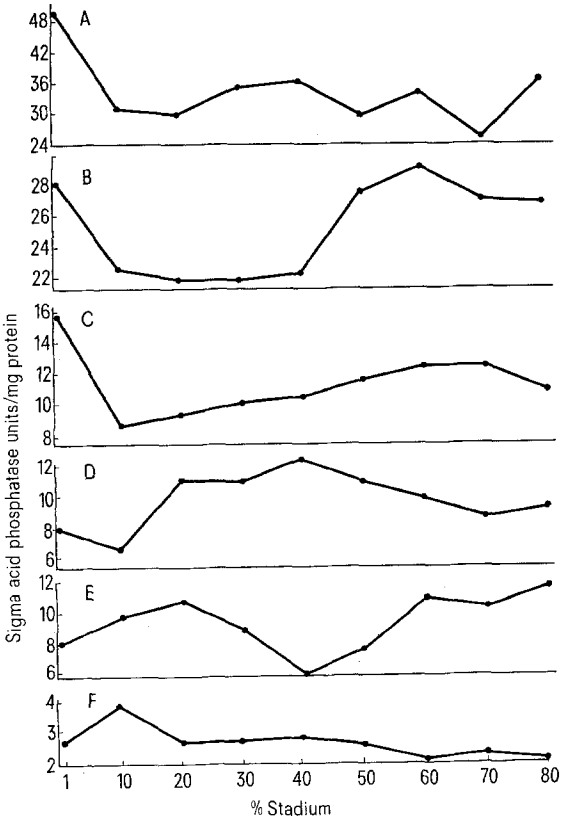
29 November 1976

Summary Acid phosphatase activity in the hemolymph and cuticle was higher during the early part of the fifth instar than at any other time. The enzyme activity in the fat body, testes, salivary glands, and midgut was statistically the same throughout the instar.

Telfer and Williams[3], Terando and Feir[4], Patel and Schneiderman[5] and Bassi and Feir[6] have shown that there are quantitative and qualitative changes in proteins during the development of the insects studied. In this paper we have concentrated on the activity changes of 1 protein, acid phosphatase, during the last larval (fifth) instar of the milkweed bug, Oncopeltus fasciatus.



Acid phosphatase activity in fifth instar bugs. *A*, midgut; *B*, fat body; *C*, cuticle; *D*, testes; *E*, salivary glands; *F*, haemolymph.

Material and methods. Newly ecdysed fifth instar male bugs were collected from the stock colonies. At 2, 24, 48, 72, 96, 120, 144, 168 and 196 h after ecdysis the males were dissected and 6 tissues (midgut, fat body, cuticle, testes, salivary glands and haemolymph) were removed for acid phosphatase activity and protein assays. The method of acid phosphatase assay was the Sigma colorimetric determination (Sigma Tech. Bul. 104) at 35 °C for 30 min and the protein was assayed by the Lowry technique[7].

Haemolymph was collected in a micropipette after severing the legs and antennae. 5 µl was collected for enzyme assay and 1 µl for protein determination. The dissection of the other tissues was done in an iced dissecting tray and the tissues were homogenized in ice cold citrate buffer (pH 4.8). Aliquants of the homogenates were used for enzyme assay and protein determination. 10 replicates of the haemolymph assays and 5 replicates of each of the other tissues were made at each time interval.

The length of the instar varied from 8 to 10 days and in order to compare measurements of bugs of the same post-ecdysis physiological age, the data were plotted on graph paper and the enzyme activity was interpolated at 10% intervals of the instar. The interpolated numbers were evaluated statistically (analysis of variance) to determine whether there were significant differences between 10% interval measurements. Then the Tukey Multiple Range Test was used to identify which 10% intervals were significantly different.

1 Acknowledgment. This research was supported in part by NSF grant GB 26542-A1 to D. F.
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