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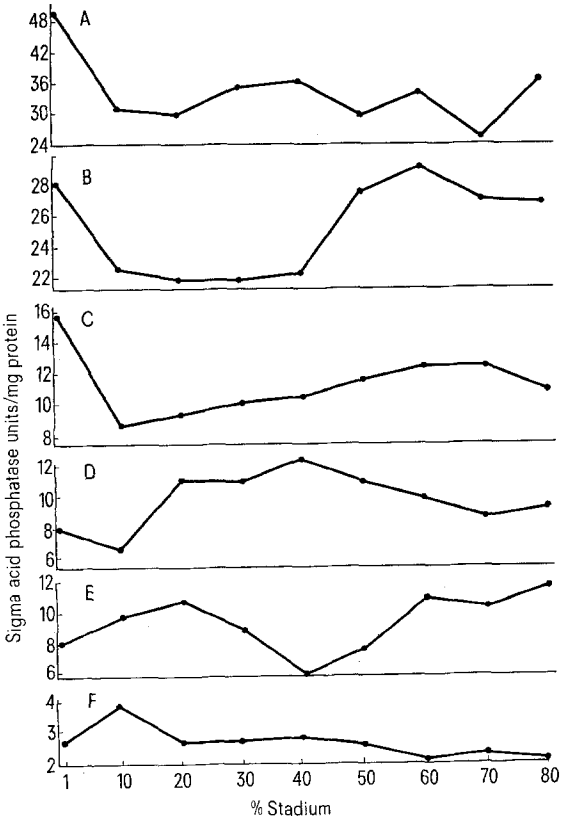
Summary Acid phosphatase activity in the hemolymph and cuticle was higher during the early part of the fifth instar than at any other time. The enzyme activity in the fat body, testes, salivary glands, and midgut was statistically the same throughout the instar.

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.

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Results and discussion. Our results (figure) showed that acid phosphatase activity in the cuticle was significantly higher ($p = 0.05$) at 1% of the instar than at 10, 20, 30, or 40% of the instar. In the haemolymph, the acid phosphatase activity was significantly higher ($p = 0.05$) at 10% of the instar than at any other time period measured. In addition, the 1, 20, 30, and 40% measurements were all significantly higher than the 60% time period. There were no significant differences between 10% interval measurements in the midgut, fat body, testes, or salivary gland tissues.

These results indicate that acid phosphatase does exhibit variation during the fifth instar of the milkweed bug in the cuticle and haemolymph tissues. The role of the enzyme and the significance of the variation in activity is not known at this time.

Combined effect of veratridine and sodium aspartate on the rabbit retinas in vitro

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Summary. Combined effect of veratridine and sodium aspartate on rabbit retinas in vitro was studied. Observations suggested that mode of action of veratridine on the PII and PIII components of ERGs was modified by the presence of sodium aspartate in perfusate.

Veratridine (mol.wt 673.81) is an alkaloid extracted from veratrum subadilla, and is known to increase the negative after-potential, to decrease the threshold potential and to induce repetitive discharge in neurons and other tissue[1]. The mode of action has been considered such that veratridine increases the permeability of sodium and potassium across an excitable cell membrane[1]. In this study, the combined effect of veratridine with sodium aspartate on perfused rabbit retinas was investigated.

Methods. Albino rabbits weighing 3 kg each were used. The electroretinogram (ERG) was recorded from in vitro retinal preparations. Techniques for excision and perfusion of the retina were the same as those described in previous reports[2,3]. Ames' solution[4] was used as an incubating medium. No blood plasma was added. The temperature of the incubating medium was maintained at 36 °C. The osmotic pressure of the medium was 305 mOsm/l. The medium was equilibrated by perfusing it continuously with a mixture of 95% O_2 and 5% CO_2 prior to and during use. This retinal preparation was shown to produce ERGs of constant amplitude over a period of 3 h[5]. The stock solutions of veratridine and sodium aspartate were added to the incubating medium in a volume ratio of 1 (or less): 100. Concentrations of chemicals were shown as those after dilution in perfusate. Chemicals reached their active site by perfusion from the ganglion cell side of the retina.

Results and discussion. Effects of veratridine on the action potential of in vitro retinas appear in 2 ways[6,7]: no effect on ERG in appearance (weak veratrinization), or de-

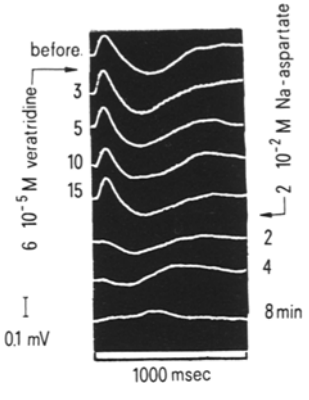

Fig. 1. Sodium aspartate of 2×10^{-2} M was administrated, 40 min after the administration of 6×10^{-5} M veratridine. The lapsing time on the right side was measured from the end of sodium aspartate administration. Responses were amplified by pre-amplifiers having a passband of 0.8–1000 Hz. Responses to 32 stimuli were summed on an averaging computer. The averaged responses were displayed on an oscilloscope and were photographed. The positivity of the active electrode, placed on the ganglion cell side of the retina, was recorded as an upward deflection. The reference electrode was placed on the receptor cell side. Stimuli were provided by a xenon flashtube (Grass PS-22) triggered by an electric stimulator. The light from the photo-stimulator was attenuated by polariod films. Radiometric measures, corrected for this attenuation, indicated that approximately 3.5×10^{-3} ergs/flash cm^2 of white light were falling at the position at which the perfused retinas were placed. The stimulus frequency was 1 flash/sec.

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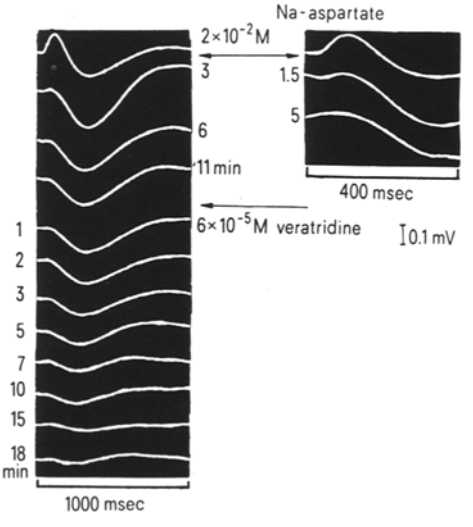

Fig. 2. To a fresh retina, sodium aspartate of 2×10^{-2} M was administrated, resulting in isolation of aspartate-insensitive PIIIs. 13 min after the administration, veratridine of 6×10^{-5} M was perfused. Analyzing times of the right and left columns were different, and were 400 and 1,000 msec, respectively. Lapsing time on the left side was measured from the end of veratridine administration.