percentage was calculated on the basis of the ratio of germinated spores to non-germinated ones. Growth yield was measured as µg dry weight/30 ml of the medium. Deoxyribose and ribose nucleic acids were estimated using diphenylamine and orcinol reagents, respectively[11]. Proteins were estimated by using Folin's phenol reagent[12]. Estimation of inorganic phosphate (Pi) was done by forming a complex with ammonium molybdate (phosphomolybdic acid) and its reduction with 1,2,4-amino-naphthol sulphonic acid (ANSA) reagent[13].

The observations presented in figure 1 show that in the presence of 0.6 mM of Ca^{2+} (optimal concentration for the growth of *T. vulgaris*), added in the culture medium, there was a higher percentage of germination of spores than in the absence of this divalent cation. The stimulatory effect of Ca^{2+} on germination was more (about 2-fold) at the initial (90 min) stage than at the following stages (105 min and 120 min) of incubation. After about 2 h it was difficult to calculate the percentage of germination because of the formation of a hyphal network and the entanglement of spores in its mesh. Ca^{2+} was found to increase mycelial yield as well as the yield of soluble proteins, DNA, RNA and free Pi by about one and a half fold over the control (fig. 2).

On the basis of these findings it can be suggested that germination is an energy-requiring process, and therefore, it is dependent on Ca^{2+} concentration. This is further supported by our earlier observation that Ca^{2+} is associated with increased hydrolysis of ATP in *T. vulgaris*[14]. Similarly to Mg^{2+} which stabilizes the nonspecific acid and alkaline phosphatases of *T. vulgaris*[15], Ca^{2+} protects its membrane-bound ATPase from thermal inactivation[8] and perhaps the most important function of Ca^{2+} is the stabilization of cell membranes of this obligate thermophile so as to protect the thermolabile components including the precursors of proteins and nucleic acids which are essential for higher mycelial yield at elevated temperature (thermophilic growth) and spore germination.

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Effect of precocene II on acid phosphatase activity in the large milkweed bug, *Oncopeltus fasciatus* (Hemiptera: Lygaeidae)

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Summary. When precocene II was applied to the fourth instar milkweed bug, there was a significant decrease in acid phosphatase activity in the hemolymph at the 40%, 50%, 60%, and 70% time periods in the stadium.

Changes in acid phosphatase synthesis and activity have been shown to be related to juvenile hormone by several workers. Bassi and Feir[1] found that application of a juvenile hormone mimic (Law-Williams, Calbiochem) to 5th instar male milkweed bugs stimulated the synthesis of acid phosphatase near the end of the stadium. They demonstrated that the synthesis was controlled at the transcriptional level[2]. Beel and Feir[3] found an increase in acid phosphatase activity in the hemolymph, salivary glands, and testis of the 5th instar male milkweed bug after treatment with a juvenile hormone mimic. Postlethwait and Gray[4] showed that ovarian acid phosphatase in the adult female *Drosophila melanogaster* was dependent on juvenile hormone levels.

The present study was undertaken to see whether the antiallatotropin, precocene II, would affect acid phosphatase activity in the 4th instar milkweed bug, *Oncopeltus fasciatus,* and thus support the role of juvenile hormone in acid phosphatase activity.

Materials and methods. 4th instar bugs within 1 h of ecdysis were collected from the stock colonies at 1-h intervals between 08.00 h and 12.00 h. The stock colonies were maintained at room temperature of approximately 25 °C and 14L:10D light cycle. After collection 25 bugs were placed in 90 × 50 mm crystallizing dishes with a moistened cotton dental roll and dried milkweed seeds. The day of collection was considered day zero of the stadium and the day when one-half or more of the bugs had undergone ecdysis was the last day of the stadium. For ease of comparing the data with the literature the data are reported in percent of stadium although the measurements were actually made at 24-h intervals.

The 4th instar bugs have endogenous juvenile hormone. Precocene acts on the corpora allata to prevent juvenile hormone from being made[5]. Precocene II was sprayed over the bottom of a petri dish with milkweed seeds at a concentration of 7.0 µg/cm^2 in acetone[5]. Control dishes were sprayed with acetone. The acetone was allowed to evaporate completely before bugs were placed in the dishes. The insects remained in the dishes for the entire stadium. At 4 h after the start of the treatment and then at 24-h

intervals hemolymph was colled from the antennae and legs of the bugs. Immediately after collection the hemolymph was assayed for protein by the method of Lowry et al.[6] and for acid phosphatase by the colorimetric method described in Sigma Bulletin No. 104 (Sigma Chemical Co., St. Louis). The specific activity of acid phosphatase is reported in Sigma units/mg protein.

The data were analyzed by a computer program, 'Analysis of variance and covariance including repeated measures'. The Tukey paired means comparison post hoc procedure was used on specific interval means across the stadium and between treatment means at specific intervals to identify that part of the stadium where a significant difference in acid phosphatase activity occurred.

Results and discussion. The pattern of acid phosphatase activity in the control bugs (table) shows low activity after ecdysis. Activity starts to increase at 30% of the stadium, reaches its peak at 60%, and then drops down again before ecdysis. Treating the bugs with precocene prevented the increase in acid phosphatase activity and in general the precocene caused a significant reduction in acid phosphatase activity at 40%, 50%, 60%, and 70% of the stadium.

These data support the hypothesis of stimulation of acid phosphatase activity by juvenile hormone and they present the pattern of acid phosphatase activity in the 4th instar milkweed bug. The role of the acid phosphatase in the milkweed bug is not known. Acid phosphatases hydrolyze phosphoric monoesters under acidic conditions and they are usually associated with lytic or degradative actions. They are also involved in the removal of protein terminal phosphates and in transphosphorylation reactions[7]. Since juvenile hormone stimulates molting to another juvenile stage there should be less degradation in its presence than in its absence when the insect molts to the adult stage. This seems to argue for a role for a non-degraditive function for the acid phosphatase stimulated by juvenile hormone. Since acid phosphatases are present in many tissues and all animals that have been studied as well as in many plants, the particular functions of acid phosphatase are of considerable biochemical interest.

Effect of precocene on acid phosphatase activity in the 4th instar milkweed bug

Percent of stadium	Acetone (sigma units/mg protein)	Precocene
10	10.9± 0.8	13.5±3.8
20	12.1± 2.1	18.8±8.3
30	15.7± 5.9[a*]	11.4±3.7
40	23.7± 4.2[a,b]	4.1±1.2[a]
50	36.7±11.8[b,c]	6.5±1.3[b]
60	48.0±24.9[c,d]	7.6±0.9[c]
70	27.4±13.0[d,e]	8.5±1.2[d]
80	3.5± 1.2[e]	9.5±1.7

Values are means ± SD.
*Data in the same column followed by the same letter are significantly different at at least the 0.05 level. Data in the same line (comparing treatments) followed by the same letter are significantly different at at least the 0.01 level.

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Harmless technique for removal of the cell coat from human spermatozoa

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Summary. Coating proteins were removed from viable human spermatozoa using a low concentration of buffered urea. Electrophoretic and ultrastructural analyses demonstrated the effectiveness of the urea treatment. Sperm motility analysis carried out before and after the procedure confirmed that this chemical method does not damage functional spermatozoal activity.

In order to carry out studies on the chemico-physical nature of the sperm surface, the cell membrane must first be made free of the adhering proteic material, i.e., the 'coat'. However, the traditional procedures followed to remove the cell coat may induce damage on the underlying structures, thereby hampering successive studies[1]. With this important aspect in mind, we developed a sperm-coat removal method using buffered urea which, because of its high dipolar moment, breaks the bonds between the external face of the plasma membrane and the coating proteins.

Materials and methods. In separate experiments, 15 normal semen samples were collected in sterile plastic reservoirs and kept at 33–25 °C until fluidification took place. At this point a phosphate-buffered saline (PBS, 0.3 M; pH 7.4) solution was added to the specimens in the ratio of 1:1 and the preparation was filtered first through at 8-µm Unipore (BioRad) filter membrane in order to trap sperm aggregates and somatic cells, and then through a 0.6-µm Unipore filter membrane on which the spermatozoa were collected and thus separated from the smaller seminal components. The spermatozoa were repeatedly flow-washed (1.0 ml/min) with PBS at room temperature until the absorbance of the collected fractions (1.0 ml) reached the base-line at 280 nm. At this point a 0.3 M urea PBS solution was put in to circulation (1.0 ml/min). Absorbing fractions were classified as 'PBS fractions' and 'urea fractions' and analyzed with a SDS-PAGE method[2]. Sperm viability was evaluated before and after the urea treatment by means of motility analysis carried out microstrobophotographically[3]. The effects of urea on the sperm surface were assessed by labeling PBS- and ureatreated spermatozoa with both ferritin-conjugated concanavalin A (ConA-F) and cationized ferritin (F^+) in order to detect glucosidic and mannosidic groups and to determine the distribution of the total negative charges[4,5]. Labeling experiments were performed at 35 °C on unfixed sperm aliquots, removed from the filter by flow