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.

1 S. Bassi and D. Feir, Comp. Biochem. Physiol. *40A*, 103 (1971).
2 S. Bassi and D. Feir, Comp. Biochem. Physiol. *41B*, 771 (1972).
3 C. Beel and D. Feir, J. Insect Physiol. *23*, 761 (1977).
4 J. Postlethwait and P. Gray, Devl Biol. *47*, 196 (1975).
5 W. Bowers and R. Martinez-Pardo, Science *197*, 1369 (1977).
6 O. Lowry, N. Rosebrough, N. Farr and R. Randall, J. biol. Chem. *193*, 265 (1951).
7 V. Hollander, The enzymes, vol. 4, 3rd edn. Academic Press, New York 1971.

Harmless technique for removal of the cell coat from human spermatozoa

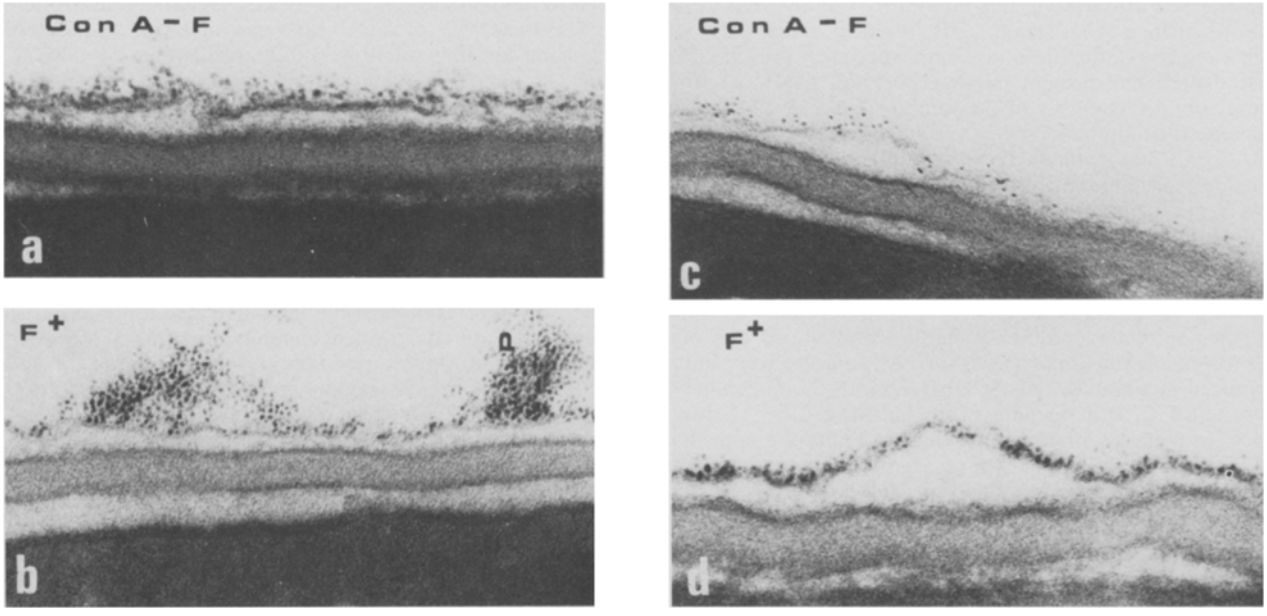
L. Silvestroni, C. Sartori, A. Modesti and G. Frajese

Institute of Clinical Medicine V, Institute of Histology, Faculty of Sciences, and Institute of General Pathology, University of Rome, I-00198 Rome (Italy), 16 October 1981

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



ConA-F and F^+ labeling distribution on head plasma membrane of PBS-washed (a and b, respectively) and urea-treated (c and d, respectively) spermatozoa. × 110,000.

inversion, using 30 µl of commercial preparations (Miles Lab.) per ml of incubation medium. Labeled cells were processed for electron microscopy and unstained sections were examined with a Philips E400 electron microscope.

Results and discussion. More than 80% of the PBS-washed spermatozoa demonstrated a rapid, straightforward progression, while after urea treatment this percentage was reduced to 70%. Seminal plasma was removed from the spermatozoa after circulation of 10 ml of PBS. As soon as the urea solution started to flow over the spermatozoa, a protein peak was observed in the filter effluent which reached the base-line after 6 ml of solution. Electrophoretic analysis showed that proteins collected from PBS fractions were identical to those collected from urea fractions. As far as the labeling experiments are concerned, PBS-washed spermatozoa bound ConA-F as well as F^+ in a patchy, outstretching pattern along the plasma membrane (fig., a and b), whereas urea-treated spermatozoa took up both the labels to a lesser degree and bound them in a more regular pattern (fig., c and d). On the basis of these results it appears that spermatozoa are easily removed from seminal fluid using synthetic membrane filters. Furthermore, it appears that the coating proteins remaining on the cell surface after PBS washing are removed by the urea treatment while cell integrity, as assessed by sperm motility analysis and by electron microscopy, remains intact. The close similarity between PBS-(seminal) and urea-(spermatozoal) released proteins suggests an external origin of the sperm coat[6]. The clear-cut differences in ConA-F and F^+ distribution before and after urea treatment confirmed the ability of urea to remove coating material from the sperm surface. In our hands, the method proposed here seems to have an edge on current techniques because it does not necessitate tryptic digestion or detergent incubation, thus preserving the integrity of spermatozoa. Finally, it provides both coatfree spermatozoa and relatively pure coat preparations suitable for subsequent studies.

1 R.C. Jones and W.V. Holt, J. Reprod. Fert. *41*, 159 (1974).
2 G. Fairbanks, T.L. Steck and D.F.H. Wallach, Biochemistry *10*, 2606 (1971).
3 A. Makler, Fert. Steril. *30*, 192 (1978).
4 G.L. Nicolson, in: Advanced Techniques In Biological Electron Microscopy, vol. 11, p. 1. Ed. J.K. Koehler. Springer, Heidelberg 1978.
5 D. Danon, L. Goldstein, Y. Marikovsky and E. Skutelsky, J. Ultrastruct. Res. *38*, 500 (1972).
6 M. Gordon, P.V. Dandekar and W. Bartoszewicz, J. Ultrastruct. Res. *50*, 199 (1975).

Lactate dehydrogenase specificity and subunit assembly in neural tissues of the teleost *Phallichthys amates*

J.S. Frankel

Department of Zoology, Howard University, Washington (D.C. 20059, USA), 25 August 1981

Summary. The tissue specificity of lactate dehydrogenase (EC 1.1.1.27) in brain and eye of the teleost *Phallichthys amates* was examined by acrylamide gel electrophoresis. It is suggested that subunit association is a function of gene product accessibility superimposed upon genetic restriction of assembly.

The tissue specific patterns of lactate dehydrogenase (lactate: NAD oxidoreductase, EC 1.1.1.27) have been investigated in numerous fish species[1–5]. In teleosts, 3 gene loci, the Ldh-A, -B, and -C loci, have been identified by immunological and biochemical techniques[6–8]. Most teleostean species exhibit high C polypeptide synthesis in derivatives of neural ectoderm, primarily in the neural retina and to a lesser extent in mesencephalon, diencephalon and optic