TABLE 1
Characterization of nuclear histones of spermatozoa

<table>
<thead>
<tr>
<th>Method</th>
<th>Spermatozoa from the</th>
<th>Spermatozoa from the</th>
<th>Spermatozoa from the</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>testes</td>
<td>seminal-vesicles</td>
<td>oviducts</td>
<td></td>
</tr>
<tr>
<td>TCA-alkaline fast green</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Protamine absent, lysine or arginine rich histones present</td>
</tr>
<tr>
<td>TCA-alkaline fast green after acetylation or deamination</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Arginine rich histones</td>
</tr>
<tr>
<td>Bromophenol-blue picric acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Arginine or Lysine rich histones</td>
</tr>
<tr>
<td>Bromophenol-blue picric acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Arginine rich histones</td>
</tr>
</tbody>
</table>

Symbol used = + = Positive reaction.

(Monne and Slaughterback, 1950), but the arginine rich histones remain unaffected.

Results and Discussion

Results of the cytochemical studies on nuclear histones of spermatozoa are summarized in Table I.

All the sperm-cells taken out from the testes reacted positively to TCA-alkaline fast green test, indicating that protamines are absent and lysine or arginine rich histones are present. Similar results were observed in all the sperm-cells teased apart from the seminal vesicles of male and oviduct of the female. Staining with Bromophenol blue also gave a positive test. Even after deamination or acetylation the sperm-cells from different reproductive organs reacted positively.

Thus spermatozoa of Mecistocephalus sp. are rich in arginine and there is no subsequent shift to protamines. None of the spermatozoa examined from different male and female reproductive parts revealed any contrast in staining.

Hence, the nature of basic nuclear protein does not indicate two different types of spermatozoa in Mecistocephalus sp.

However it is remarkable to note that in mature spermatozoa there is a transition from somatic or lysine rich to arginine rich histones, but there is no further alteration to protamines. Similar results were obtained in Drosophila sperm-cells by Das et al. (1969). It will be interesting to find the stage at which the transition occurs from lysine rich to arginine rich during the spermatogenesis.

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ACTIVATION OF PROPHENOLOXIDASE IN THE HEMOLYMPH OF CALLIPHORA VICINA R-D

PHENOLOXIDASE (E.C. 1.10.3.1 : O-Diphenol : Oxygen oxido-reductase) is present in the hemolymph of Calliphora vicina R-D in an inactive form, the phenoloxidase. It is activated by a cuticular activator present in the 3rd instar larva or by an endogenous activator present in the hemolymph. However, sometimes activation takes place spontaneously, in some insects at certain age, or by treatment with proteolytic enzymes, metal ions, alcohols and acetone. Therefore, experiments were carried out to find out whether the phenoloxidase of C. vicina also undergoes induced activation, by such substances.

Preparation of phenoloxidase, cuticular activator (for control) and determination of phenoloxidase activity were done according to standard methods, except for some modifications. The pellet obtained after 144,000 g centrifugation was dissolved in 5 ml phosphate buffer (0.05 M, pH 6.5) and 100 microlitre of it were mixed with 4 ml of Dopa (2-4-Dihydroxyphenylalanine)
mg/ml of phosphate buffer) and 100 microlitre of cuticular activator (control) or the testing substance (acetone, alcohol, proteolytic enzyme, metal ion salts 0·01 M). The color development was measured in terms of O.D. using mercury filter of 492 m\(\mu\) and 20 mm cells in Eppendorf spectrophotometer, while the rate of dopa oxidation was measured at 35° C for 10 minutes and was recorded by an automatic recorder. Enzyme blanks were also run and found non-reactive, during preliminary experiments.

![Graph showing effect of alcohols and acetone on dopa oxidation rate](image1)

**Fig. 1.** Effect of alcohols and acetone on the activation of prophenoloxidase in comparison with and without activator.

Experiments showed (Fig. 1) that acetone produced slight activation while ethyl alcohol and butyl alcohol produced significant activation. Methyl and propyl alcohols had no effect. Such an activation has been reported for other insects\(^{12-13}\). However, activation by ethyl and butyl alcohols only and not by methyl and propyl alcohols is strange. It may be due to some structural affinity.

Figure 2 indicates that trypsin activated the prophenoloxidase while pepsin did not. Activation by alpha-chymotrypsin and dipeptidase has been reported in Calliphora\(^2\), while by alpha-chymotrypsin in the case of Bombyx mori\(^1\). Although pepsin and trypsin both are proteolytic, the former had negligible effect. It may be due to the difference in the optimum pH. This also indicates that there exists proteolytic mechanism for prophenoloxidase activation but under certain optimum conditions.

Activation of prophenoloxidase by metal ions has been reported by a few workers\(^0\)\(^-\)\(^1\). The role of cupric ion has been discussed in the case of silkworm\(^1\). During the present investigations it was observed that NaCl, MgCl\(_2\), MnCl\(_2\), and MnSO\(_4\) did not activate the prophenoloxidase (Fig. 3), although, Mg\(^{++}\) and Mn\(^{++}\) increase the activity of many enzymes. However, FeCl\(_3\), CuCl\(_2\), FeSO\(_4\) and CuSO\(_4\) activated the prophenoloxidase to a great

![Graph showing effect of pepsin and trypsin on dopa oxidation rate](image2)

**Fig. 2.** Effect of pepsin and trypsin on the activation of prophenoloxidase in comparison with hemolymph only and hemolymph + activator.

![Graph showing effect of different salts of metal ion on dopa oxidation rate](image3)

**Fig. 3.** Effect of different salts of metal ion on the activation of prophenoloxidase in comparison with hemolymph extract (prophenoloxidase) and the hemolymph + activator.
extent. It confirms the findings of Ashida and Naqi and Ahmed. Ashida has discussed in detail, the probable role of cupric and cuprous ions in the activation mechanism while Naqi and Ahmed biochemically showed the presence of a cupric or ferric ion dependent isozyme of diphenoloxidase, in polyacrylamide gels. Moreover, the presence of copper in the insect blood and the decrease in the diphenoloxidase activity (one-third) due to conversion of cupric form to cuprous in the silkworm hemolymph, on standing, indicates some role of these ions in the prophenoloxidase activation mechanism, at least on one isozyme.

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A SPONTANEOUS ALLOTETRAPLOID ALOE

The genus Aloe L. forms the largest group amongst the three major genera of the succulent plants belonging to the tribe Aloinae. In India, aloe is mostly cultivated as a vegetative ornamentals being easily propagated through suckers. While studying the chromosomes of the tribe Aloinae, Brandham has recently analysed 113 species of Aloe, and recorded 106 diploids (2n = 2x = 14), one triploid (2n = 3x = 21), five tetraploids (2n = 4x = 28) and a hexaploid (2n = 6x = 42). In addition, there are some previous reports of polyploidy in Aloe, and Reston has recorded a pentaploid (2n = 5x = 35) of the Aloe ciliaris known only in cultivation. Sharma and Mallick have recorded a triploid of A. humilis, while Snod has recorded three hexaploid species of Aloe. Thus, the total known polyploids among 113 species of Aloe is 12, i.e., the genus approximately shows 10-6% incidence of polyploidy.

The present plant is a new addition to the list of polyploids in Aloe and is perhaps the first case of spontaneous allotetraploidy in the Aloinae. This plant shows features of some of the morphological characters. It is unique in having a globose condensed raceme type of inflorescence with greenish-yellow flowers, while all the tetraploids known so far have long racemes with red flowers (Brandham, personal communication). Root tip squashes made using Brandham's technique clearly show 2n = 4x = 28, comprising 16 long and 12 short chromosomes (Fig. 1). Following the classification of the long chromosome pairs in the Aloinae by Snod, the 4L chromosomes appear to be of two different lengths (Fig. 1, hexagons and squares represent two sets of homologues), indicating that the chromosome complement comprises two different genomes. The plant thus appears to be an allotetraploid and was probably a hybrid at the

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Fig. 1. Photomicrograph of somatic metaphase showing 2n = 4x = 28 = 16 L + 12 S. Hexagons and squares show homologous L and S chromosome sets in the complement. Magnification, × 1,740.