### Table 1: Stability constants bivalent metal chelates of $H_2NE (\mu = 1\cdot0 \text{ M } NaClO_4)$

<table>
<thead>
<tr>
<th>Metal-chelate</th>
<th>25°C</th>
<th>30°C</th>
<th>35°C</th>
<th>$-\Delta G^0$ K. cal/mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (II)</td>
<td>log $K_1$</td>
<td>11.401 (0.001)</td>
<td>11.421 (0.001)</td>
<td>11.452 (0.002)</td>
</tr>
<tr>
<td></td>
<td>log $K_2$</td>
<td>3.851 (0.001)</td>
<td>3.871 (0.001)</td>
<td>3.902 (0.002)</td>
</tr>
<tr>
<td>UO$_2$ (II)</td>
<td>log $K_1$</td>
<td>11.601 (0.001)</td>
<td>11.842 (0.002)</td>
<td>12.102 (0.002)</td>
</tr>
<tr>
<td></td>
<td>log $K_2$</td>
<td>4.001 (0.001)</td>
<td>3.971 (0.001)</td>
<td>3.952 (0.002)</td>
</tr>
<tr>
<td>VO (II)</td>
<td>log $K_1$</td>
<td>12.101 (0.001)</td>
<td>12.232 (0.002)</td>
<td>12.352 (0.002)</td>
</tr>
<tr>
<td></td>
<td>log $K_2$</td>
<td>4.301 (0.001)</td>
<td>4.271 (0.001)</td>
<td>4.251 (0.001)</td>
</tr>
</tbody>
</table>

The deviations in the experimental and computational values are shown in parenthesis.

The above sets of titrations were also performed at 30°C and 35°C and values of log $K_1$ and log $K_2$ were obtained. Different methods were used to compute the stability constants and the values so obtained were found in agreement. The theoretical formation curves of these metal chelates at 25°C were derived by using average log $K_1$ and log $K_2$ values, as 11.40 and 3.85 for Cu(II) chelate; 11.60 and 4.00 for UO$_2$(II) chelate; and 12.10 and 4.30 for VO(II) chelate, respectively. A perusal of the experimental and theoretical curves indicates negligible deviations. The experimental stabilities and their deviation from computational values along with the free energy changes are shown in Table I. The stability constants are in the sequence VO(II) > UO$_2$(II) > Cu(II).

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**ESTRAGOL—A PERSISTENT DEPOLARISING AGENT OF THE SKELETAL MUSCLE MEMBRANE**

The effect of Estragol, (Fig. 1; inner square) a substance isolated from the essential oil of Feronia Limonia was studied on the isolated sartorius semitendinosus and rectus abdominis muscles of frog (Rana tigrina). The muscles were suspended in an isolated organ bath containing aerated frog Ringer solution and Isotonic contractions were recorded with gimbals lever giving a 12 fold magnification.

Estragol caused contractions of the sartorius and the semitendinosus muscles when added in a concentration of $3 \times 10^{-3}$ M. Initial contraction was followed by relaxation, even when the drug was present in the bath (Fig. 1, a and b). It caused contraction of the rectus abdominis of frog when added in the same concentration. This effect was markedly reduced in a K$^+$ ($9 \times 10^{-2}$ M)$^2$ depolarised muscle (Fig. 1, a) and in the presence of quinidie (5.1 $\times 10^{-4}$ M, 10 mts.) or Mn$^{3+}$ ($1 \times 10^{-2}$ M, 10 min.). It reduced the responses to K$^+$ (4 $\times$ $10^{-2}$ M, 2 mts.) on isolated frog rectus muscle (Fig. 1, c).
The nature of the effect of Estrogen on sartorius and semitendinosus muscles, reduction of its effect in K+ depolarized muscle and in the presence of quinidine or Mn+2 and its ability to reduce the action of K+ indicate that it produces its effect by persistent depolarisation of the muscle membrane. This effect is interesting, since it is produced by a substance having a chemical structure that is different from the known membrane depolarising agents.

Fig. 1. (a) Isolated Sartorius muscle of frog (Rana tigrina). Effect of Estrogen (at E, $3 \times 10^{-3}$ M, 5 mins.) in normal and potassium depolarised (at K $9 \times 10^{-2}$ M) muscle. (b) Effect of Estrogen (at E) on isolated Semitendinosus muscle of frog. (c) Isolated Rectus abdominis of frog—II. Effect of Estrogen (at E) on potassium induced (at K $4 \times 10^{-2}$ M, 2 mins.) contractures. (d) Effect of quinidine (at Q, $5.1 \times 10^{-4}$ M, 10 mins.) and managanese (at M, $1 \times 10^{-2}$ M, 10 mins.) on Estrogen (at E) induced contractures.

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EFFECT OF CAFFEINE ON TESTICULAR HYALURONIDASE OF DIFFERENT SPECIES

Hyaluronidase is widespread in the semen of mammals and it has been suggested that the enzyme may play a role in the passage of spermatozoa through the zona pellucida and by modifying the structure of this membrane to make it more susceptible to the action of another sperm-borne enzyme acrosin which is widely believed to be involved in the penetration process. The hyaluronidase activity of semen sample correlates with the number of sperm and hyaluronidase in seminal plasma has been shown to originate from sperm acrosome. Various authors have reported an increase in fertilizing capacity of spermatozoa on addition of this enzyme. The present communication describes the in vitro effect of caffeine on testicular hyaluronidase of different species.

For standard hyaluronidase assay weighed testicular tissues from rat and goat were homogenized (1:9 w/v) in ice-cold 0.1 M sodium-phosphate citrate buffer pH 4.5. Cetyltrimethyl-ammonium bromide was added to a final concentration of 0.1% prior to homogenization in order to release the enzyme from its particulate bound form. The homogenate was allowed to stand in ice for 30 minutes and was then centrifuged at 105,000 g for 60 minutes at 4° in a VAC 60 ultracentrifuge. The clear supernatant obtained was used as the enzyme source. Human semen was frozen for 30 minutes and seminal plasma was removed as supernatant by centrifugation at 2,000 g for 15 minutes at 4°. The spermatozoa were washed and homogenized in 0.1 M sodium-phosphate citrate buffer, pH 4.5, as detailed above. Ovine testicular hyaluronidase was obtained from V.P. Chest Institute, New Delhi.

Hyaluronidase activity was assayed by the rate of release of free N-acetylglucosamine from hyaluronic acid by a modification of the colorimetric methods of Reissig et al. and Bollet et al. Protein concentration was determined by the method of Lowry et al. using crystalline bovine albumin as the standard.

Table I depicts the in vitro effect of 0.5 mmol/l caffeine on testicular hyaluronidase activity. With the exception of acrosomal hyaluronidase from human semen caffeine activates rat, goat and ovine testicular e-enzyme. Acrosomal enzyme was moderately inhibited to the extent of 14%. The order of hyaluronidase activation was

Ovine testis > goat testis > rat testis.

An increase in substrate concentration did not produce any change in the extent of caffeine inhibition or activation on hyaluronidase activity thereby suggesting that it probably acts by attaching to some allosteric site on the enzyme protein. Recently rabbit and bull acrosomal hyaluronidases have been shown to possess properties similar to the testicular enzyme.