TABLE I

Stability constants bivalent metal chelates of H_2NE ($\mu = 1{\cdot}0\ M\ NaClO_4$)

Metal-chelate		Stability constant			$-\Delta G°$ K.cals/mole		
		25° C	30° C	35° C	25° C	30° C	35° C
Cu (II)	$\log K_1$	11·401 (0·001)	11·421 (0·001)	11·452 (0·002)	20·79	21·20	21·64
	$\log K_2$	3·851 (0·001)	3·871 (0·001)	3·902 (0·002)			
UO_2 (II)	$\log K_1$	11·601 (0·001)	11·842 (0·002)	12·102 (0·002)	21·27	21·93	22·62
	$\log K_2$	4·001 (0·001)	3·971 (0·001)	3·952) (0·002)			
VO (II)	$\log K_1$	12·101 (0·001)	12·232 (0·002)	12·352 (0·002)	22·37	22·88	23·40
	$\log K_2$	4·301 (0·001)	4·271 (0·001)	4·251 (0·001)			

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

to the stepwise formation of 1 : 1 and 1 : 2 species as shown by the equations :

$$\tfrac{1}{2}\,M^{2+} + H_2NE + OH^- \rightarrow \tfrac{1}{2}\,[MNE]^0 + \tfrac{1}{2}\,H_2NE + H_2O \quad (5)$$

$$\tfrac{1}{2}\,(MNE)^0 + \tfrac{1}{2}\,H_2NE + OH^- \rightarrow \tfrac{1}{2}\,[M(NE)_2]^{2-} + H_2O \quad (6)$$

The above sets of titrations were also performed at 30° and 35° C and values of $\log K_1$ and $\log K_2$ were obtained. Different methods[5] 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[1] and Isotonic contractions were recorded with gimble lever giving a 12 fold magnification.

Estragol caused contractions of the sartorius and the semitendinosus muscles when added in a concentration of 3×10^{-3} M. Initial contraction was followed by relaxation, even when the drug was present in the bath (Fig. 1, *a* and *b*). It caused contracture of the rectus abdominis of frog when added in the same concentration. This effect was markedly reduced in a K^+ (9×10^{-2} M)[2] depolarised muscle (Fig. 1, *a*) and in the presence of quinidine ($5{\cdot}1 \times 10^{-4}$ M, 10 mts.) or Mn^{++} (1×10^{-2} M, 10 min.). It reduced the responses to K^+ (4×10^{-2} M, 2 mts.) on isolated frog rectus muscle (Fig. 1, *c*).

The nature of the effect of Estragol on sartorius and semitendinosus muscles, reduction of its effect in K^+ depolarised muscle and in the presence of quinidine or Mn^{+3} 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[4].

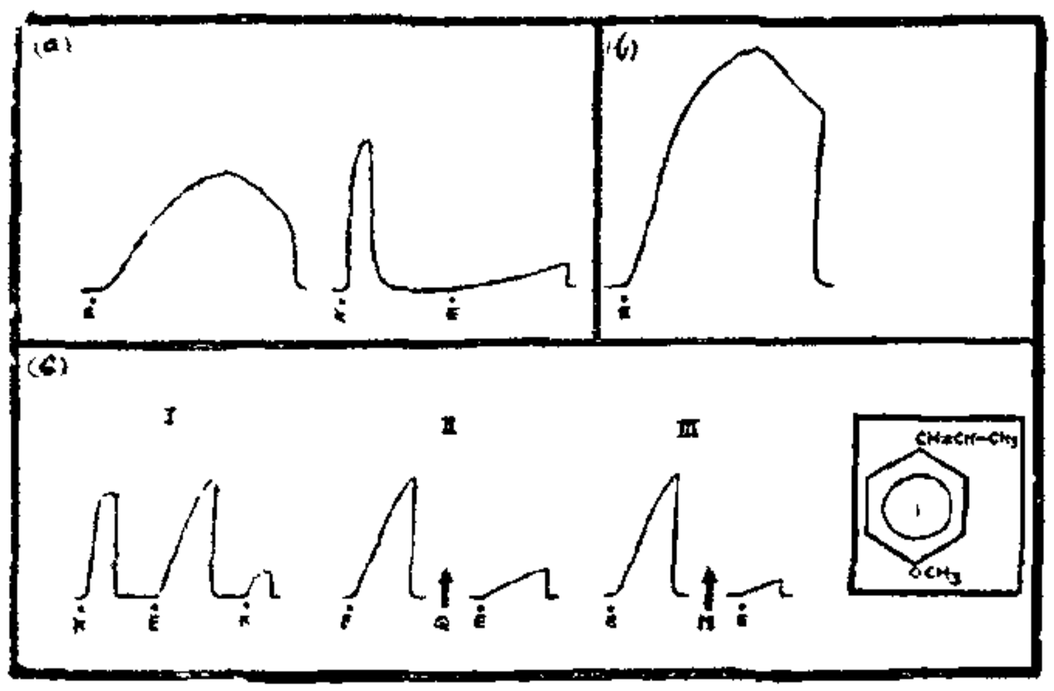

FIG. 1. (*a*) Isolated Sartorius muscle of frog (*Rana tigrina*). Effect of Estragol (at E, 3×10^{-3} M, 5 mts.) in normal and potassium depolarised (at K 9×10^{-2} M) muscle. (*b*) Effect of Estragol (at E) *on* Isolated *Semitendinosus muscle of frog.* (*c*) Isolated Rectus abdominis of frog—I. Effect of Estragol (at E) on potassium induced (at K 4×10^{-2} M, 2 mts.) contractures. II and III. Effect of quinidine (at Q, $5{\cdot}1 \times 10^{-4}$ M, 10 mts.) and managanese (at M, 1×10^{-2} M, 10 mts.) on Estragol (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 pelluciaa[1, 2] 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[3, 4]. The hyaluronidase activity of semen sample correlates with the number of sperm[5] and hyaluronidase in seminal plasma has been shown to originate from sperm acrosome[6]. Various authors have reported an increase in fertilizing capacity of spermatozoa on addition of this enzyme[7,8]. 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.[9] The homogenate was allowed to stand in ice for 30 minutes and was then centrifuged at 105,000 xg 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 xg for 15 minutes[10] 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.*[11] and Bollet *et al.*[12]. Protein concentration was determined by the method of Lowry *et al.*[13] using crystalline bovine albumin as the standard.

Table I depicts the *in vitro* effect of 6·5 mmol/l caffeine on testicular hyaluronidase activity. With the exception of acrosomal hyaluronidase from human semen cafleine activates rat, goat and ovine testicular 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[14].