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].

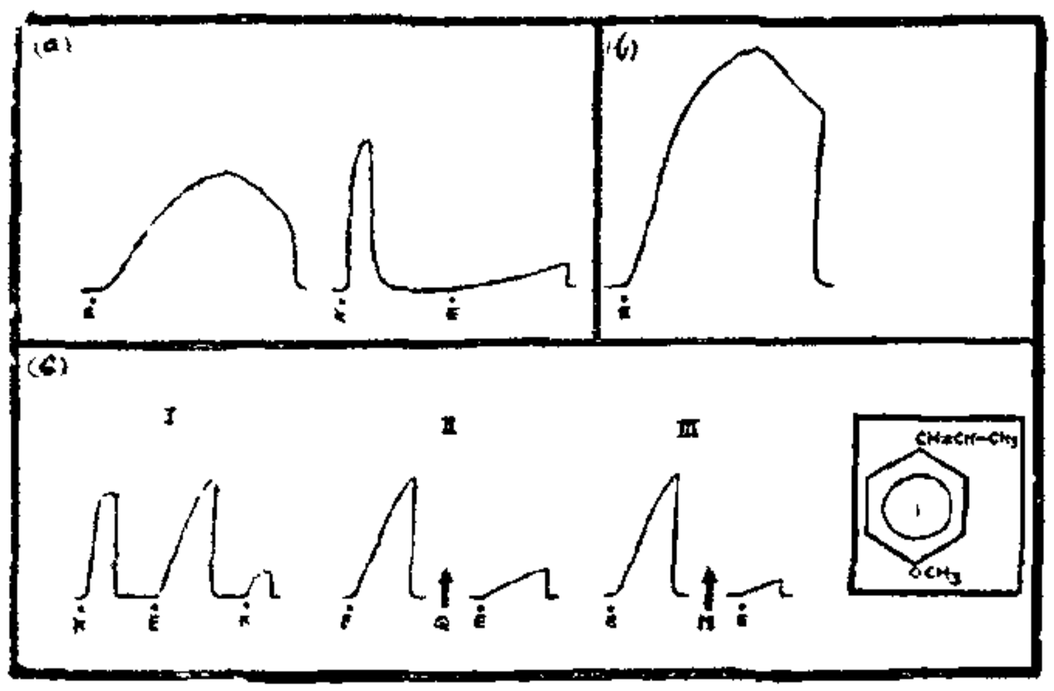

FIG. 1. (*a*) Isolated Sartorius muscle of frog (*Rana tigrina*). Effect of Estragol (at E, 3×10^{-3} M, 5 mts.) in normal and potassium depolarised (at K 9×10^{-2} M) muscle. (*b*) Effect of Estragol (at E) *on* Isolated *Semitendinosus muscle of frog.* (*c*) Isolated Rectus abdominis of frog—I. Effect 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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1. *Staff Department of Pharmacology*, University of Edinburg, E and S, Livingstone, Ltd., London, 1968, p. 2.
2. Evans, D. H. L., Schild, H. O. and Theselff, H. L., *J. Physiol.* (*London*), 1958, 143, 474.
3. Chiarandini, D. J. and Stefani, E., *Ibid.*, 1973, 232, 129.
4. Koelle, G. B., *The Pharmacological Basis of Therapeutics*, 3rd Edition, Edited by Goodman, L. S. and Gillman, A, The Macmillan, Co., New York, p. 598.

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].

TABLE I

in vitro *comparison of the effect of caffeine* (6·5 mmol/1) *on testicular hyaluronidase activity*

Species	Origin	% inhibiton (↓) or activation (↑) (mean ± S.D.)
Rat	testis	21·87±1·55 ↑
Goat	testis	51·65±0·84 ↑
Ovine	testis	235·76±0·02 ↑
Human	acrosome	14·38±0·66 ↓

Specific activity = μ moles of N-acetyl glucosamine liberated/mg. protein/minute.

In contrast to the above report the present study indicates that human acrosomal hyaluronidase appears to be different from rat, goat and ovine testicular enzyme with respect to its inhibition by caffeire.

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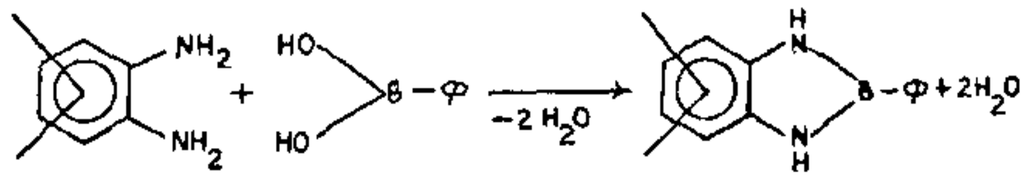
1. Stambaugh, R. and Buckley, J., *J. Reprod. Fertil.*, 1969, **19**, 423.
2. Hartree, E. F., *Of Microbes and Life*, eds., J. Monod and E. Borek, Columbia University Press, New York. 1971, p. 271.
3. Stambaugh, R., Brackett, B. G. and Mastroianni, L., *Biol. Reprod.*, 1969, **1**, 223.
4. Zaneveld, L. J. D., Robertson, R. T. and Williams W. L., *FEBS Letters*, 1970, **11**, 345.
5. Werthessen, N. T., Berman S., Greenberg, B. E. and Gargill, S. L., *J. Urol.*, 1945, **54**, 565.
6. Swyer G. I. M., *Biochem., J.*, 1947, **41**. 413.
7. Rowlands, I. W., *Nature*, 1944, **154**, 332.
8. Kurzrok, R., Leonard, S. and Conrad, H., *Amer. J. Med.*, 1946, **1**, 491.
9. Kumar Parveen, *M.Sc. Thesis*, Panjab University, 1972.
10. Roussel, J. D. and Stallcup, O. T., *J. Reprod. Fertil.*, 1966, **12**, 423.
11. Reissig, J. L., Strominger, J. L. and Leloir, L. F., *J. Biol. Chem.*, 1955, **217**, 959.
12. Bollet, A. J., Bonner, W. M. Jr. and Nance, J. L., *Ibid.*, 1963, **238**, 3522.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. K. and Randall, R. J., *Ibid.*, 1951, **193**, 265.
14. Zaneveld, L. J. D., Polakoski, K. L. and Schumacher, G. F. B., *Ibid.*, 1973, **248**, 564.

SYNTHESIS OF SOME NEW BENZODIAZABOROLE DERIVATIVES

BENZODIAZABOROLE, pyrimidine and a few purine type boron analogues where —C(—H)— group in heterocyclic ring is replaced by boron were prepared and screened for treatment of cancer[1,2] with certain success.

Liao *et al.*,[3] have prepared and tested heterocyclic boronic acids for the same purpose. The application of boron heterocyclics as stabilizers, antimetabolite, disinfectants and chemosterilants have also been recorded.

In view of this, a number of benzodiazaboroles have been prepared. Their structures have been confirmed by elemental analyses and recorded I.R. frequencies. Heterocyclic boron compounds reported herein have been prepared by conventional methods, *i.e.*, azeotropic distillation of the reactants to remove the water formed in the reaction.



Their physical properties have been recorded in Table I. These compounds are iso-electronic with indole and show only a small absorption due to a dipolar B–N, B–O or B–S bonds. Non ionic form is the predominant of the two resonating structures.

Preparation of Borimidazolines :

Equimolar amounts of the aryl boronic acid (0·01 mol) and *o* phenylenediamine (0·01 mol) were taken in xylene and heated under reflux for 4–5 hours. The water was removed azeotropically and the solvent xylene was distilled off at reduced pressure. The products were crystallized from carbon tetrachloride or benzene.

The infrared spectra of these borimidazoline compounds show the presence of the important functional groups. All show the characteristic NH-streching absorption in the regions of 3450 — 3400 cm^{-1}, aromatic -CH- at 2950 — 2900 cm^{-1} and B-N group, being double bond character appears in the region of 1520 — 1380 cm^{-1}. The absorptions for N-H bands in KBr are very strong and sharp at 3470 cm^{-1} and 1430 cm^{-1}, trivalent bornon at 1350 cm^{-1} and out of plane C-H modes of boron substituted aromatic rings at 760 750 cm^{-1}, 700 690 cm^{-1}.