Effects of ethylene dimethanesulphonate on reproductive function in rats

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Ethylene dimethanesulphonate (EDS), a diester of ethylene glycol and methane sulphonic acid, belongs to the homologous series of esters of general formula: $(CH_2)_n(OSO_2CH_3)_2$. Unlike other members of the series, which suppress bone marrow activity, EDS appears to have no such action, at least in rats and mice, although showing inhibitory activity against tumours. Its antispermatogenic effects also differ strikingly from the homologues and involve the intermediate range of spermatogenic cells—meiotic spermatocytes and spermatids (Jackson, 1966), whereas related diesters predominantly attack spermatogonia in the early stages of spermatogenesis. However, repeated oral courses of EDS (5×25 mg/kg orally) at 5 week intervals eventually produce irreversible sterility in rats.

Again, in the rat, EDS produces marked inhibition of the accessory sexual structures (prostate and seminal vesicles), indicating pharmacological action on the androgenic function of the Leydig cells. This may be central or peripheral in origin and is not a feature of the antispermatogenic action of the homologous diesters. Single injections of EDS have now been observed to produce spermatoceles in the epididymis within a few days, which may be important in relation to antifertility action of this compound. These lesions are cystic spaces evidently formed by rupture of the thin wall of the tube, leading to extravasation of fluid containing sperm. These spermatoceles may be visible to the naked eye or microscopic and multiple but there is no evidence that vascular damage is the cause. It is tempting to infer that Leydig cell damage is responsible since the epididymis is androgen-dependent. Only one other organic compound, α-chlorohydrin (U-5897) has been implicated in the production of epididymal damage and spermatocele formation (Ericsson & Baker, 1969; Ericsson, personal communication).

The question is whether the various changes induced in the reproductive tract by EDS are due primarily to interference with androgen control or result from more than one pharmacological activity.

REFERENCES

ERICSSON, R. J. & BAKER, V. F. (1969). Male antifertility compounds: biological properties of U-5897 and U-15,646. *J. reprod. Fert.*, in the Press.

JACKSON, H. (1966). *Antifertility Compounds in the Male and Female.* Springfield: Charles C. Thomas.

The occurrence of pharmacologically active substances in, and the action of drugs on, preparations of the sea anemone *Actinia equina*

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Mathias, Ross & Schachter (1960) found histamine, tetramethylammonium and a pharmacologically active polypeptide in extracts of *Actinia equina*. They were unable to demonstrate the presence of 5-hydroxytryptamine or esters of choline, but their extraction and bioassay techniques may have led them to overlook small amounts of these substances. Indeed, it is known that the choice of solvent system may influence the recovery of choline esters (Aprison, 1967). In this study, therefore, five different solvent systems have been used for the extraction of anemones and the active substances identified by chromatographic, colorimetric, fluorometric and bioassay methods. In general the results of Mathias *et al.* (1960) have been confirmed

in that histamine, tetramethylammonium and an active polypeptide were easily detected; the substances were qualitatively distributed as they had described. Despite repeated and careful extractions of large quantities of anemones (up to 100 g) we have failed to detect 5-hydroxytryptamine (assay limit 10 ng/g), acetylcholine, propionylcholine or butyrylcholine (assay limits 10, 60 and 80 ng/g respectively). A spot resembling tryptamine was noted in many chromatograms, as were spots of high R_F having choline ester-like activity on the guinea-pig ileum; these latter spots have not yet been identified. Noradrenaline, dopamine and dopa were also found (Carlyle, 1969).

The actions of drugs and electrical stimulation were measured on isolated ring preparations of the supra-oral sphincter, suspended from a pair of platinum hooks in sea-water at 15° C. Contractions were elicited by the application through the platinum hooks of square wave pulses of 35 v and 0·1–1·0 msec duration at various frequencies. Contractions were recorded isotonically or isometrically. Both fast and slow contractions were obtained as previously reported by Ross (1957) for preparations from *Calliactus*. Surprisingly, and unexpectedly, responses were often obtained to single shocks in fresh preparations.

The following drugs were without effect on the resting tone, spontaneous activity or responses to electrical stimulation of the isolated preparations; acetylcholine, carbachol, methacholine, eserine, neostigmine, nicotine, atropine, hyoscine, curare, suxamethonium, hemicholinium and choline in concentrations of up to 10^{-2} g/ml.

Noradrenaline, dopamine and isoprenaline in concentrations of up to 5×10^{-3} g/ml. were similarly ineffective, as were a variety of α or β-blocking agents, tryptamine and 3-hydroxytryptamine. Cocaine (10^{-3} g/ml.) caused a prolongation of the response to electrical stimulation. The above results are essentially similar to those reported for *Calliactus* and *Metridium* by Ross (1960), who also found that reserpine was without effect. However in these experiments reserpine (10^{-7}–10^{-6} g/ml. for 12–36 hr) caused changes in the excitability of intact anemones and isolated preparations, as first reported by Pearce (personal communication, 1968). The effects of reserpine may, in many experiments, be reversed by noradrenaline or dopamine. Catecholamines may play some part in neuromuscular activity in the sea anemone.

REFERENCES

APRISON, M. H. (1967). In HOLMSTEDT, BO., Mobilisation of acetylcholine by cholinergic agents. *Ann. N.Y. Acad. Sci.*, **144**, 433–458.

CARLYLE, R. F. (1969). The occurrence of catecholamines in the sea anemone *Actinia equina*. *Br. J. Pharmac.*, **36**, 182P.

MATHIAS, A. P., ROSS, D. M. & SCHACHTER, M. (1960). The distribution of 5-hydroxytryptamine, tetramethyl-ammonium, hormarine and other substances in sea anemones. *J. Physiol., Lond.*, **151**, 296–311.

ROSS, D. M. (1957). Quick and slow contractions of the isolated sphincter of the sea anemone *Calliactus parasitica*. *J. exp. Biol.*, **34**, 11–28.

ROSS, D. M. (1960). The effects of drugs and ions on neuromuscular preparations of sea anemones. II, On sphincter preparation of *Calliactus* and *Metridium*. *J. exp. Biol.*, **37**, 753–774.

Isolation of a new oxytocic peptide from bovine posterior pituitary lobes

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Oxytocin and vasopressin, the polypeptide hormones of the neural lobe of the bovine pituitary gland, can readily be dissociated from the neurophysins and