METHODS AND MATERIALS

(A) NICOTINIC GANGLIONIC BLOCKERS :

DOG NICTITATING MEMBRANE :

Mongrel dogs of either sex weighing 9-19 kg were
anaesthetised with 500 mg/kg of urethane and 50 mg/kg of
chloralose in combination or 100 mg/kg of chloralose administered
intravenously. Subsequently small doses of the anaesthetic agents
were used whenever required. Starting from the superior cervical
ganglion, the pre-ganglionic cervical sympathetic nerves of both
sides were identified and separated from the vagus nerves. The
preganglionic nerve trunk was placed over a pair of shielded
platinum electrodes. The nerve was cut well below the electrode.
An INCO stimulator was used for stimulation of the preganglionic
nerve. Supramaximal rectangular pulses (3 times the maximal) of
2.5 msec duration, at frequencies ranging from 1 to 250/sec for
30 sec were employed. The stimuli were applied every 10 min.
Contractions of both the nictitating membranes were recorded in
each animal with a balsa wood frontal writing lever. The load on
the lever was 3.5 g at the horizontal equilibrium position and
the contractions were magnified 10-fold. The contractions were
recorded on a smoked drum. Frequency response-curves were
plotted on a graph paper with percentage of the maximal
contraction on the ordinate and log of the frequency on the
abscissa.
Each ganglion blocker was used in four doses and 3 experiments were set up for each dose. After eliciting a control panel of responses, the ganglion blocker was given intra-arterially and 15 min later, responses to nerve stimulation were re-elicited. After recovery from ganglionic blockade, responses to another dose of the ganglion blocker were elicited. It was possible to record the effects of only two doses of a ganglion blocker with one membrane since reproducible control responses could not, in general, be elicited subsequently. Since responses of both the membranes were recorded in the same animal, it was possible to obtain responses with all the four doses of a blocker in one experiment.

**Intra-arterial administration of the drugs:**

A polyethylene tube of a fine bore was passed into the common carotid artery of the dog through the superior thyroid artery. The tip of the polyethylene tube in the artery was coaxed in the direction of the flow of blood. Into the other end of the tube was inserted a 21 gauge needle attached to a three way stop cock. All the drugs were administered into the common carotid artery through the three way stop cock and were followed by 0.5 ml of normal saline every time. The external carotid artery above the carotid bifurcation and all the branches of the common carotid artery except the internal carotid artery were ligated.

All the doses of the antagonists were administered by the intra-arterial route.
Young adult rabbits weighing 1.2 - 2.5 kg and guinea pigs weighing 250 - 400 g were killed with a sharp blow at the back of the neck and were exsanguinated by opening the neck vessels. The abdomen was opened, and 2.5 to 3 cm of the terminal part of ileum near the ileo-caecal junction was removed. Tyrode solution of the following composition: NaCl, 8.0 g; KCl, 0.2 g; CaCl₂, 0.2 g; MgCl₂, 0.2 g; NaHCO₃, 1.0 g; NaH₂PO₄, 0.05 g; dextrose, 1.0 g; and double distilled water up to 1 litre was used. With two ml pipette filled with Tyrode solution and kept at an angle of 30° into the lumen, at one end, the contents of the lumen were gently washed out.

The ileum was suspended in a 33 ml organ bath, maintained at 37 °C ± 1 °C and bubbled with oxygen in the case of guinea pig ileum and with air in the case of rabbit ileum. The contractions of the rabbit ileum were recorded with isotonic and auxotonic levers and those of the guinea pig ileum with isotonic lever. The isotonic and auxotonic levers were subjected to 1 g tension and the magnification was 10-fold. The recording was made on smoked kymograph paper. The preparations were allowed to stabilize for 20 to 30 min before the start of the experiment. The preparations were regularly washed every 7-8 min except during the exposure to the blocker. Two preparations from the same ileum were set up simultaneously.
Nicotine (5.55 x 10⁻⁸, 1.85 x 10⁻⁷, 5.55 x 10⁻⁷, 1.85 x 10⁻⁶, and 5.55 x 10⁻⁶) and dimethylphenylpiperazinium (DMPP) (1.5 x 10⁻⁵, 3.0 x 10⁻⁶, 6.0 x 10⁻⁶, 7.5 x 10⁻⁶, and 1.5 x 10⁻⁵) were used to obtain control responses with the rabbit ileum. Nicotine (1.80 x 10⁻⁷, 2.88 x 10⁻⁷, 4.5 x 10⁻⁷, 5.76 x 10⁻⁷, 9.0 x 10⁻⁷, and 1.80 x 10⁻⁶) and DMPP (1.5 x 10⁻⁵, 3.75 x 10⁻⁶, 4.8 x 10⁻⁶, 7.5 x 10⁻⁶, and 1.5 x 10⁻⁵) were used to obtain control responses with the guinea pig ileum. The contact time for nicotine and DMPP was 30 sec. Dose-response curves were constructed by plotting log dose of the agonist on the abscissa and percentage of the maximal height of contraction on the ordinate.

For testing the antagonistic effects of the ganglion blockers, each blocker was kept in the bath for 15 min before the addition of the agonist. Not more than two doses of a blocker were tested in one preparation. Since two preparations from the same ileum were set up simultaneously it was possible to test all the four doses on the same ileum. Three preparations were employed for each dose. Recovery of control responses to the agonists occurred after 15-20 min of wash-out of hexamethonium and tetraethylammonium and the lower two doses of mecamylamine, pempidine, chlorisondamine and pentolinium. With the higher two doses of the latter four blockers, recovery occurred after 50-70 min of wash-out.
The preparations were mounted according to the method of Hukovic (1961). Male guinea pigs weighing 250 - 500 g were sacrificed by a blow on the back of the head and bled to death. The animals were fixed with their backs on the dissecting board; the abdomen was opened by a midline incision and the gut was displaced to the right. The testis was pushed into the abdominal cavity by pressure on the scrotum. Holding each testis, the vas deferens was freed from the connective tissue and dissected from the epididymis. The testis was then removed. Grasping the cut end of the vas deferens with a small forceps, it was separated from the adjacent tissue. Without further cleaning of the vas deferens, the hypogastric nerve was identified. The right and the left nerves could be easily seen in the middle of mesentery of the colon. The nerve was tied and cut 5 cm from the vas deferens and cleaned to within 0.5 cm of the vas. The remainder of the nerve (upto the vas deferens) which was fine and diffuse was preserved by isolating along with the piece of peritoneum which contained it. During the dissection, the organ and the nerve were moistened with Kreb's solution of the following composition: NaCl, 6.60 g; KCl, 0.35 g; CaCl₂, 0.28 g; KH₂PO₄, 0.182 g; MgSO₄·H₂O, 0.294 g; NaHCO₃, 2.1 g; dextrose, 2 g; and double distilled water up to 1 liter. The vas deferens was then cut from the urethra and mounted together with its nerve in a bath of 33 ml capacity containing Kreb's solution bubbled with carbogen.
(95% oxygen and 5% carbon dioxide) and maintained at 32 °C. Two preparations from the same animal were set up simultaneously.

The proximal end of the vas deferens was tied to the oxygen tube and the distal end was attached to a balsa wood writing lever which wrote on a smoked paper. The lever gave 10-fold magnification and the load on the lever was 0.5 g at the horizontal equilibrium position. The hypogastric nerve was passed through shielded platinum electrodes dipping into the bathing fluid. The preparations were stimulated by an INCO stimulator supramaximally (as in the case of dog nictitating membrane) with rectangular pulses of 2.5 msec duration at variable frequencies (1, 2, 5, 5, 7, 5, 10, 25, 50, 100/sec and up to 250/sec) for 30 sec every 10 min.

For testing the inhibitory effects of the ganglion blockers each blocker was kept in the bath for 15 min before starting stimulation of the hypogastric nerve and remained in the bath thereafter. Only two doses of a ganglion blocker were tested on one preparation. The bath fluid was changed every 7-8 min except during exposure to the ganglion blocker. Since 2 preparations from the same animal were set up simultaneously it was possible to get a complete dose-response curve on preparations from the same animal. Three preparations were employed for each dose of the blocker. Recovery from block occurred 15-20 min after the lower two doses of each blocker and 50-70 min after the higher two doses of each blocker.

Frequency-response curves were constructed by plotting
the log of frequency of nerve stimulation on the abscissa and per centage of the maximal contraction on the ordinate.

(B) NON-NICOTINIC GANGLIONIC BLOCKER:

CAT NICTITATING MEMBRANE:

Cats of either sex weighing 2-3.5 kg were anaesthetised with chloralose (70 mg/kg, intravenously) after preliminary ethyl chloride anaesthesia. A fine polyethylene cannula was inserted into the lingual artery as described for the dog nictitating membrane. All the drugs under test were administered intra-arterially. The external carotid artery was occluded for 15 sec at the time of administration of all the drugs. The contractions of both the membranes were recorded on smoked drum with isotonic lever (10-fold magnification) subjected to 3.5 g tension at the horizontal equilibrium position.

$4-(\text{m-chlorophenylxan}-\text{oxy})-2$-bulytrimethylammonium chloride (MoM-A-343) \((6.09 \times 10^{-7}, 1.22 \times 10^{-7}, 2.03 \times 10^{-7}, 4.06 \times 10^{-7})\) and muscarine \((5.75 \times 10^{-8}, 1.44 \times 10^{-8}, 2.37 \times 10^{-8}, 5.75 \times 10^{-8})\) were used as the agonists.

To test the nature of antagonism of atropine, dose-response curves of muscarine and MoM-A-343 were plotted before and after four different doses of atropine. To avoid tachyphylaxis, muscarine and MoM-A-343 were administered at intervals of 15 to 20 min. The effect of each dose of atropine was studied in triplicate. The plan of experiments was similar to that described for the dog nictitating membrane. Atropine block
lasted for about 20 min with all the doses studied.

**DRUGS:**

Nicotine, 1,1-Dimethyl-4-phenylpiperazinium iodide (DMPP), hexamethonium chloride, tetraethylammonium bromide, mecamylamine hydrochloride, pemipidine tartrate, chlorisondamine dimethochloride, pentolinium tartrate, muscarine, 4-(m-chlorophenylcarbamoxy)-2-ethyltrimethylammonium chloride (McN-A-343), and atropine sulphate were used throughout the study. Unless otherwise specified, doses of the drugs are expressed in terms of μg/ganglion for dogs and cats and in terms of M(mole/litre) for the rabbit and guinea pig ileum and for the guinea pig hypogastric nerve vas deferens.

Statistical tests are those described by Snedecor (1956).