METHODS AND MATERIAL :

Isolated guinea pig ileum.

Guinea pigs of either sex weighing 300 to 500 g were used. The animals were starved overnight for 18 hr before sacrificing them for dissecting out the ileum. The isolated ileum was suspended in Tyrode solution of the following composition: NaCl 8.0 g; KCl 0.2g; $CaCl_2$ 0.2 g; $MgCl_2$ 0.1 g; NaH_2PO_4 0.05 g; $NaHCO_3$ 1.0 g and glucose 1.0 g per litre. The solution was maintained at 37°C and bubbled with carbogen gas (O_2 95% and CO_2 5%). The contractions were recorded by an isotonic frontal writing lever at 1-2 g tension. The lever magnification was ten fold.

Cumulative responses to acetylcholine were recorded. The response to each dose of acetylcholine was allowed to develop for 20 sec before adding the next dose. A rest period of 9 min was given after the wash.When two successive dose-response curves were alike the tissue was exposed to the test compound for a period of 2 min before the redetermination of acetylcholine dose-response curves. Thus the interval between two successive determinations was maintained constant (9 min).

Isolated frog rectus abdominis muscle :

The rectus abdominis muscle of the frog (Rana tigrina) was set up in frog Ringer solution in 25 ml organ bath (Burn,1952). The frog Ringer solution used was of the following composition: NaCl 6.5 g; KCl 0.14 g; $CaCl_2$ 0.12 g; $NaHCO_3$ 0.2 g; and glucose 1.0 g per litre. The solution was maintained at room temperature (30 °C) and bubbled with air. The contractions were recorded by an isotonic frontal writing lever at 1 - 1.5 g tension. The lever system employed gave ten fold magnification. The muscle was allowed to stabilise for a period of 3 hr.

Cumulative responses to different doses of acetylcholine were recorded. The contractions were allowed to develop for 3 min after administration of each dose and a rest period of 6 min was given after the wash. When two successive dose-response curves were reproducible, the tissue was exposed to the test drug. Each dose of the test drug was added 3 min before the redetermination of acetylcholine dose-response curves. Thus the interval between two successive determinations was always kept constant (6 min).

Isolated rabbit hearts.

Rabbits of either sex weighing 1.5 - 2.5 kg were used. The isolated rabbit hearts were set up according to Langendorff technique (Perry 1965). The hearts were perfused by Locke solution and the contractions were recorded with Starling heart lever. The composition of Locke solution was : NaCl 9.0 g; KCl 0.42 g; $CaCl_2$ 0.24 g; $NaHCO_3$ 0.5 g and glucose 1.0 g per liter. The solution was vigorously bubbled with carbogen gas. The solution was warmed to 37°C before perfusing through the hearts and the rate of perfusion was 10 $\pm$ 2 ml/min.

After setting up, the hearts were allowed to stabilise for a period of 30 min. The drugs were injected close to the glass cannula into the perfusion solution. The drugs were injected at an interval of 6 min.

Isolated rabbit aortic strip.

Rabbit aortic strip was set up as described by Furchgott and Bhadrakom (1953). The aortic strip was mounted in Kreb bicarbonate solution in a 5 ml organ bath at 37°C and bubbled with carbogen gas. The composition of the Kreb bicarbonate solution was : NaCl 6.87 g; KCl 0.49 g; $MgSO_4$ 7 H_2O 0.14 g; $CaCl_2$ 0.28 g; NaH_2PO_4 0.14 g; $NaHCo_3$ 2.1 g; and

glucose 2.0 g per litre.The tissue was allowed to stabilise for one hour before commencing the experiments. The lever magnification was fourteen fold.

The responses to a given dose of adrenaline or noradrenaline was recorded for 3 min. Then a rest period of 15 min was given after the wash before recording the next response. Different concentration of the antibiotics and the degradation products were added 3 min before redetermining the amine responses.

Rabbit ear artery perfusion.

The central ear artery of the rabbit was perfused as described by De la Lande and Rand(1965). The artery was perfused with Kreb bicarbonate solution with the help of a self priming peristaltic pump. The rate of perfusion was maintained constant at 10 $\pm$ 2 ml/min. The temperature of the perfusion solution was kept constant at 37°C. The perfusion pressure was recorded by means of a Condon's mamometer. An equilibration period of 2 hr was allowed before giving the drugs. Responses to adrenaline and noradrenaline were recorded every 2 min. The antibiotics and degradation products were dissolved in Kreb bicarbonate solution and perfused for 20 min before redetermination of the responses to the amines.

Experiments in cats.

Seventy two healthy cats of either sex weighing 2.0 to 4.5 kg were used. Anesthesia was induced with ether and maintained with chloralose (80 mg/kg iv). The blood pressure was recorded from the left common carotid artery on smoked kymograph paper by a Condon type of mercury manometer for big animals (U.S.P. XV 1955). Heparin (2000 units) was injected in the arterial cannula to avoid clotting of the blood. The cats were maintained on artificial ventilation.

The basal blood pressure of the cat was allowed to stabilise for a period of 45 min. After this time, cats whose blood pressure ranged between 120-140 mm Hg were used for the experiments.

Injections were made through a glass cannula inserted into the right femoral vein. Drugs were injected in a volume of 0.1 ml/kg and were washed in with 2 ml of 0.9 per cent sodium chloride solution.

Vagal stimulation.

The right vagus nerve was dissected out and prepared for electrical stimulation. The nerve was stimulated at frequencies of 10-20 Hz (8 volts; 0.5 m sec duration) for 10 sec.

Nictitating membrane.

The nictitating membrane was set up as described by Gokhale et al.(1967). The contractions of the membrane in response to preganglionic stimulation of the superior cervical ganglion were recorded by an isotonic writing lever. The lever system employed gave twelve fold magnification.The membrane was placed under 5 g tension. The membrane was allowed to stabilise for 45 min before the stimuli were applied.

Experiments in dogs.

Blood pressure.

Twenty one healthy mongrel dogs of either sex weighing 10-15 kg were used in the experiments. The animals were anaesthetized by the intravenous administration of pentobarbitone sodium (30 mg/kg) dissolved in 0.9 per cent sodium chloride solution. Anaesthesia was maintained by injecting 5 mg/kg of pentobarbitone sodium as required. The blood pressure was recorded from the left common carotid artery as described in expmiments with cat.

The vagus nerve and preganglionic superior cervical fibres were stimulated similar to that described under cat experiments.

Method for studying the nature of antagonism.

The degree of antagonism was calculated from the dose-response curves with acetylcholine in terms of the dose ratio, which is the ratio of the equiactive doses of the agonist after and before the addition of the antagonists (Gaddum et al. 1955).

The log of the dose ratio was given by the horizontal distance between the two dose response curves. The pA_2 values of the antagonist (antibiotics and the degradation products) were determined as described by Arunlakshana and Schild(1959).

Preparation of Streptidine and Streptamine :

Streptidine.

Streptidine used in these experiments was prepared in the laboratory by the hydrolysis of streptomycin sulphate as described by Peck et al. (1945). Streptomycin sulphate (100 g) was dissolved in (500 ml) methanolic sulphuric acid (2 N solution) which was maintained at 40°C for 48 hr. Streptidine sulphate obtained as white crystals was separated by filtration and washed free from sulphuric acid by methanol followed by cold distilled water. The crystals were dissolved in

boiling N/10 sulphuric acid and allowed to recrystalise in cold. The pure crystals so obtained (47.0 g) were dried under vacuum on phosphorous pentoxide.

Pure streptidine sulphate obtained was dissolved in N/10 sulphuric acid and stochiometric quantity of barium-chloride dissolved in water was added to the solution. Streptidine sulphate was converted into hydrochloride by double decomposition. Barium sulphate was separated by filtration and the aqueous solution concentrated under vacuum to a small volume (50 ml). Five volumes of dry acetone was added. Precipitated streptidine hydrochloride was separated by filtration, dissolved in methanol and recrystalised. The crystalline material so obtained was dried under vacuum over phosphorous pentoxide. The dry substance (37.2 g) was stored in a desiccator containing phosphorous pentaoxide.

The purity of the substance was assessed by estimation of total nitrogen which was found to be 22.95 per cent against the theoretical value of 23.33 per cent. The spot test (Fiegel 1954) and chemical assay for barium were negative.

Streptamine.

Streptamine was obtained by the alkaline hydrolysis of streptidine sulphate as described by Carter et al. (1949). Streptidine sulphate (94.0 g) was dissolved in 5 N lithium hydroxide (550 ml) and refluxed for 40 hr. Precipitated lithium carbonate was separated by filtration and the filtrate concentrated to a small volume (50 ml) under vacuum. The pH of this concentrate was adjusted to 2.0 by adding concentrated sulphuric acid. Streptamine sulphate which was precipitated was separated by filtration, washed free from sulphuric acid and lithium sulphate and recrystallised.

Pure streptamine sulphate so obtained (34.0 g) was converted to streptamine hydrochloride by reacting with barium-chloride in a manner similar to that described for streptidine sulphate.

The total nitrogen content was 10.74 per cent (10.14 per cent theoretical value) and the tests for barium ions (spot test and chemical analysis) were negative.

Drugs.

Streptomycin sulphate (Chas Pfizer and Co.); kanamycin sulphate (Meiji Seika Kaisha, Japan);

viomycin sulphate (Ciba Basel); neomycin sulphate (Chas Pfizer and Co.); paromomycin sulphate (Parke Davis, India); adrenaline acid tartarate (BDH); noradrenaline acid tartarate (Burroughts Wellcome, England); histamine acid phosphate (BDH); 4-(m-chlorophenyl carbamoyloxy)-2-butynyl - trimethyl ammonium chloride (McN-A-343) (McNeil Laboratories U.S.A.); 1,1-dimethyl-4-pehnylpiperazinium iodide (DMPP) (Aldrich Laboratories U.S.A.); hexamethonium bromide (Aldrich Laboratories U.S.A.); acetylcholine chloride (Aldrich Laboratories U.S.A.); atropine sulphate (E.Merk, Dramstadt); mepyramine maleate (May and Baker London) and N-diethyl-aminoethyl-N--isopentyl-N'N'-di-isopropylurea (P.286) (Pitman Moore U.S.A.).

The doses of histamine, adrenaline and noradrenaline refer to the base and those of the other drugs refer to the salt.

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Figure No.1

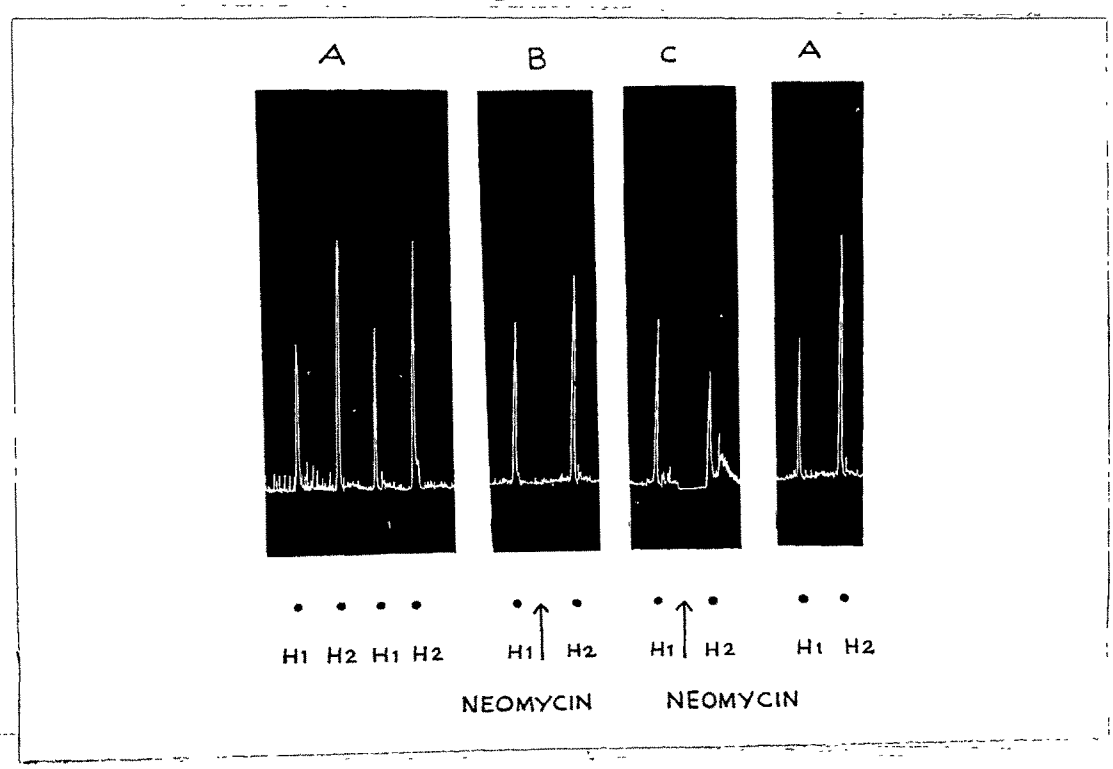

Fig.1: Isolated guinea pig ileum suspended in Tyrode solution (5 ml bath, 37°C). Responses to histamine 50 ng/ml and 100 ng/ml at H_1 and H_2 respectively. Panels A show control responses. Responses shown in panels B and C were obtained after the addition of neomycin 100 µg/ml and 1 mg/ml respectively. Panel C also shows the effect of neomycin (1 mg/ml). Time cycle 3 min.