METHODS AND MATERIALS.

Rabbit Aortic Strip:

The method of preparation of strip was essentially similar to that described by Furchgott and Bhadrakom (1953).

Rabbits of both sexes weighing 1.5 to 2.0 kg were stunned and then bled by cutting the vessels of the neck. The chest was opened and the descending thoracic aorta was removed and placed in a petri dish containing Krebs - bicarbonate solution of the following composition: sodium chloride 6.88 g; potassium chloride 0.42 g; calcium chloride 0.28 g; magnesium chloride 0.112 g; sodium dihydrogenate 0.18 g; sodium bicarbonate 2.1 g; dextrose 1.8 g and distilled water upto 1 litre at room temperature.

Excess fat and connective tissue were trimmed off. The whole length of aorta was then cut along a close spiral. During the cutting, which was done with a small, sharp-pointed scissors, the uncut end of the aorta was gradually rotated and moved forward toward the scissors in such a manner as to permit a continuous spiral incision. The resulting strip was usually 2-3 mm wide. From this strip shorter strips of 3 to 3.5 cm. length (unstretched) were cut for use in experiments. During the whole procedure of preparing the strips, the tissue was kept

moistened with Kreb's solution.

The strips were mounted in a 40 ml organ bath and tied to an isotonic frontal writing lever that placed the muscle under 4 g of tension at the horizontal equilibrium position. The bathing solution was Kreb's bicarbonate solution. The solution was maintained at 37^{o} to 38^{o} and 5% carbon dioxide in oxygen was bubbled through the solution in both bath and reservior. The preparation was left for 2 hr before testing.

Dose response curves for adrenaline and noradrenaline were obtained by cumulative administration of increasing concentrations of the amines, allowing the contraction to develop fully after each administration (3 min). Dose-response relationship for adrenaline and noradrenaline were determined in replicate on each strip prior to the administration of chlorpromazine. In studies on antagonism, chlorpromazine was placed in the bath 15 min before administration of catecholamines and remained in bath thereafter.

Thirty such preparations were set up.

Receptor-protection in rabbit aortic strip:

The selective receptor protection technique of Furchgott (1954) was used. Spiral strips from young rabbits were prepared and mounted in a 40 ml organ bath as described above.

For every experiment two preparations from the same aorta were suspended each in an individual organ bath. Atn the start of the experiment control responses to adrenaline or noradrenaline were obtained from each of the preparations. Then both preparations were exposed to the same concentration of chloropromazine but one was protected throughout the entire period of exposure (6 min) by a high concentration of adrenaline or noradrenaline which was added to the organ bath 6 min before addition of chlorpromazine. Both the protected and the unprotected strips were then repeatedly washed at 15 min intervals for a period of 90 min. Both the preparations were now tested for their sensitivity to adrenaline or noradrenaline by addition of the control dose of the stimulant drugs at 15 min intervals.

Six such experiments were performed.

Rat seminal vesicle:

Seminal vesicles were set up according to the method of Litch, Liebig and Haley (1954). Male albino rats weighing 125 to 150 g were stunned with a blow on the head and then bled to death by cutting the neck vessels. The seminal vesicles and associated structures were removed and placed in a solution of the following composition: sodium chloride 9 g, potassium chloride 0.42 g, calcium chloride 0.06 g, sodium bicarbonate 0.5 g, dextrose 0. 5 g and distilled water upto 1 litre. Coagulation

glands contained in the same connective tissue sheath were removed. Extraneous tissues were trimmed off.

The seminal vesicles were mounted in a 10 ml organ bath and attached to a frontal writing lever and contractions were recorded on a smoked drum. The preparation was bathed in a continuous flow of the bathing medium run at a rate of 15 ml per min. Flow was interrupted when stimulant drugs were added to the bath. The bathing solution was continuously oxygenated with a mixture of 95% O_2 and 5% CO_2.

Adrenaline or noradrenaline was added at six minute intervals and the response was recorded for 30 sec. Four different doses of adrenaline or noradrenaline were used for constructing a dose-response line.

In testing the blocking effect of chlorpromazine the drug was dissolved in the bathing medium perfusing the preparation. Dose-response curves for adrenaline and noradrenaline were redetermined 10 min after the change of the perfusion fluid (from chlorpromazine free to one containing chlorpromazine). Five different concentrations of chlorpromazine were employed.

Thirty such preparations were set up.

Isolated rabbit auricle:

Rabbit auricles were set up according to the technique of Burn (1952). Male rabbits weighing 1.5 to 2.0 kg were

killed by a sharp blow to the back of the neck and then bled by cutting the vessels of the neck. The chest was opened and the heart was quickly removed and placed in Locke oxygenated solution. The auricles were carefully separated from the ventricles, other adhering tissues and fat. The auricles were mounted in a 40 ml organ bath. Oxygenated Locke heart solution of the following composition was used as the bathing medium; Sodium chloride 9 g, potassium chloride 0.42 g, calcium chloride 0.24 g, sodium bicarbonate 0.15 g, dextrose 1.0 g and distilled water upto 1 litre. The solution was maintained at 29°C (± 0.5° C)

Movements of auricles were recorded on a smoked drum by means of a Starling heart lever. The preparation was allowed to stabilize for 90 min before the testing was begun. Effect of adrenaline was studied for 2 min at 15 min intervals. After obtaining reproducible responses to adrenaline, chlorpromazine was placed in the bath 10 min before the next addition of adrenaline and remained in the bath thereafter.

Six such preparations were set up.

Cats:-

Cats of either sex weighing 2 to 3 kg were used. Anaesthesia was induced with ethyl chloride and maintained with chloralose (60 to 80 mg per kg intravenously). Blood

pressure was recorded with a mercury manometer connected to the left carotid artery. Intravenous injections were made through a polyethylene cannula inserted into a femoral vein.

Rabbits:

Rabbits of either sex weighing 1.5 to 2.0 kg were anaesthetized with urethane (1.5 g per kg subcutaneously). Blood pressure was recorded with a mercury manometer connected to the left carotid artery. Intravenous injections were made through a polyethylene cannula inserted in the right external jugular vein.

Drugs: Cocaine hydrochloride, hexamethonium chloride, chlorpromazine hydrochloride, ($\pm$) - noradrenaline hydrochloride and isoprenaline hydrochloride were used throughout the expriments. The doses refer to the salts. Adrenaline base was dissolved in 0.9% saline immediately before injection.