D I S C U S S I O N :

The results of the present investigations on the isolated guinea pig ileum and the isolated rabbit heart indicated that the antibiotics, streptomycin, kanamycin, viomycin, neomycin and paromomycin differed from the degradation products, streptidine and streptamine in their pharmacological actions.

The antibiotics (1 µg/ml and above) inhibited the spontaneous contractions of the guinea pig ileum. The inhibition was transient in nature as the tissue regained its spontaneous contractions on washing the antibiotic from the bath. The contractile responses to acetylcholine were blocked. The slope value of log (dose ratio -1) versus -log B plots for all the antibiotics were not different from the theoretical value of unity for competitive antagonism, indicating that these compounds antagonised acetylcholine competitively. Taking pA_2 values of the antibiotics as a measure of potency, streptomycin and neomycin were found to be the most potent while viomycin was the least potent. Kanamycin and paromomycin were intermediate in their potency. Popovici *et al.* (1967) reported that streptomycin

contracted the uterine muscle of the guinea pig suspended in glucose free Tyrode solution. This effect of streptomycin was completely blocked by atropine indicating a acetylcholine-like action of streptomycin. The present study, however, fails to confirm the observation of Popovici et al.(1967). This could probably be due to tissue differences or to differences in the composition of Tyrode solution. They also reported that in higher doses, streptomycin inhibited the spontaneous contractions of the uterine muscle.

Unlike the inhibitory effects of the antibiotics, streptidine and streptamine both had stimulant action on the guinea pig ileum. In lower concentrations (10 µg/ml and less) these compounds enhanced the spontaneous contractions of the ileum, while in higher doses, streptamine induced a contractile response. Streptidine, in high doses did not induce contractile response. The stimulant action of both the compounds was blocked by atropine (10 ng/ml) and hexamethonium (10 µg/ml) indicating parasympathomimetic action.

Although, streptidine enhanced the spontaneous contractions of the guinea pig ileum, in higher doses it antagonised the response to acetyl-

choline. The slope value of log (dose ratio -1) versus -log B plot was less than the theoretical value of unity in this case. The pA_2 value of streptamine could not be estimated because of its spasmogenic action.

In low doses, the antibiotics did not affect the isolated rabbit heart, while in high doses(5mg) the heart was depressed. Leaders et al. (1960) have reported similar observation. They demonstrated that in higher doses, streptomycin produced an atrio-ventricular block of the isolated rabbit heart.

The degradation products elicited a positive inotropic actionon the isolated rabbit heart. This was not observed with isolated hearts obtained from reserpine treated rabbits indicating that the stimulant action was mediated through a release of catecholamines.

It is interesting to note that streptidine and streptamine both had an acetylcholine-like action as observed with the guinea pig ileum and an indirect sympathomimetic action as demonstrated with the isolated rabbit heart. Both these actions seem to be masked by the sugar residues as in the

case of streptomycin, kanamycin, neomycin and paromomycin or the amino acid residues as in the case of viomycin.

The antibiotics and streptidine antagonised the acetylcholine responses of the frog rectus abdominis muscle. In the case of the antibiotics, the slope value of log (dose ratio -1) versus -log B plots were not different from the theoretical value of unity indicating that the compounds antagonised acetylcholine competitively. The pA_2 values indicated that viomycin was the most potent and streptomycin and paromomycin were the least potent. In the case of streptidine the slope value of log (dose ratio -1) versus -log B was less than the theoretical value of unity indicating that the antagonism was not competitive. Streptamine potentiated the response of the frog rectus abdominis muscle to acetylcholine.

Brazil and Carrado (1957) demonstrated the neuromuscular blocking action of streptomycin and correlated this with the respiratory distress produced by streptomycin clinically. Subsequently the neuromuscular blocking action of kanamycin (Corrado et al. 1959), neomycin (Timmerman, 1959), paromomycin (Adamson et al. 1961) and viomycin (Brazil

et al. 1961) was also demonstrated. These observations indicated that though the molecular structures of the antibiotics were different from each other, all of them elicited neuromuscular blocking action. Brazil et al. (1959) also demonstrated that streptidine and streptamine, the degradation products of streptomycin possessed potent neuromuscular blocking action.

Brazil and Corrado (1957) characterised the neuromuscular blocking action of streptomycin as Mg^{++} - like, mediated through removal of Ca^{++} ions from the vicinity of the myoneural junction. Subsequent investigations demonstrated that the neuromuscular blocking action of the antibiotics and their degradation products could be mediated through different mechanisms of action. For example, Brazil et al. (1959) demonstrated that streptidine had neuromuscular blocking action similar to d-tubocurarine while the neuromuscular blocking action of streptamine and streptomycin was identical in nature but different from that of streptidine. In denervated tibialis anticus muscule of dog (Brazil, 1960), Ca^{++} increased the depression of the muscle preparation due to streptomycin while the contractile response to acetylcholine and potassium chloride

was depressed by streptomycin. Brazil (1960) characterised this effect of streptomycin as due to stabilisation action. Iwatsuki et al. (1958) demonstrated that streptomycin antagonised the action of succinylcholine and decamethonium while it potentiated the effect of d-tubocurarine. They have proposed that it is due to competitive inhibition of acetylcholine at the myoneural junction. A similar mechanism of action has been proposed for kanamycin by these workers.

Elmquist and Josefesson (1962) have shown that neomycin inhibits the release of acetylcholine at the myoneural junction and causes a neuromuscular block. This finding was corroborated by Brazil and Francheschi (1969). However, the dose of neomycin used by the latter authors was very high (300 µg/ml). It appears that the antibiotics and the degradation products inhibit the acetylcholine action at the myoneural junction by multiple mechanisms and produce a neuromuscular block. In the present experiments with frog rectus-abdominis muscle, streptamine however caused potentiation of the acetylcholine responses. Thus the neuromuscular blocking action of streptamine does not seem to be mediated through an antiacetylcholine action, but some other mechanism.

The chemical studies on the antibiotics indicated that though they are derived from different micro-organisms they have a common chemical core. Thus streptidine in the case of streptomycin and 2-deoxystreptamine in the case of the other antibiotics contain guanido groups. Streptamine and 2-deoxystreptamine both have mesoconfiguration.The application of Reevs cuprammonium method confirmed that the absolute stereochemistry of these two compounds is analogous (Dyer and Todd, 1963).

The antibiotics and their degradation products possess identical effects such as the neuromuscular blocking action, the antiacetylcholine action on the guinea-pig ileum and the frog rectus--abdominis muscle (except streptamine) and ototoxicity. Perhaps these common effects are contributed by the idential chemical constitution of streptamine and 2-deoxystreptamine. The addition of sugar residues or amino acid residues (viomycin) to the guanido groups does not alter their effects one way or the other.This also suggests that the toxic reactions of the antibiotics could mainly be accounted for by the central core.

The potentiation by streptamine of the responses to acetylcholine of frog rectus-abdominis

muscle was not seen with streptidine. Perhaps the additional amino groups masked this action and also contributed to the antiacetylcholine action of streptidine. The sugar residues presumably stabilises the antiacetylcholine action and makes them competitive antagonists. The contractile response of the guinea-pig ileum to streptidine was also less than that to streptamine and the antibiotics exerted antiacetylcholine action which was competitive in nature.

The antibiotics, streptomycin, kanamycin, viomycin, neomycin and paromomycin (20 mg/kg) produced a sharp and an abrupt fall of blood pressure in anaesthetized cats.The vasodepressor response to the antibiotics (20 mg/kg) was not blocked by atropine (1 mg/kg) and mepyramine (1 mg/kg) indicating that the response was not cholinergically or histaminergically mediated. The sharp and abrupt fall in blood pressure could be due to direct action of the antibiotics on the heart demonstrated in the present study. Leaders et al. (1960) have reported a similar effect of streptomycin with the isolated rabbit heart. Yamasaki (1954) has reported vasodilator response of the perfused rabbit ear with streptomycin. He had also demonstrated that

the vasodepressor response to streptomycin in rabbit blood pressure was not histamine-like and was not blocked by atropine. Thus direct vasodilation may also account for the sharp and abrupt fall in blood pressure.

Repeated injections of 10 and 20 mg/kg of the antibiotics every 10 min induced an insidious fall in blood pressure which at the end of 60 min became steady at 40 per cent of the control level. The rate of fall in blood pressure in response to 20 mg/kg of the antibiotics was greater than that due to 10 mg/kg. Repeated injections of 10 mg/kg at 30 min intervals however did not affect the blood pressure upto 3 hr.

When the antibiotics were injected repeatedly every 10 min, they probably produced a cumulative action as suggested by incomplete recovery. The insidious fall in blood pressure induced by 10 and 20 mg/kg of the antibiotics injected every 10 min could be accounted for by their effects at the myoneural junction (Brazil and Corrado 1957; Adamson et al. 1960 and 1961; Corrado and Ramos, 1960; Brazil et al. 1961). The neuromuscular blocking agents are known to produce hypotension by diminishing the respiratory excursions and

producing apnoea. Kubikowski and Sczreniswki (1963) have reported similar findings with streptomycin (15 mg/kg). They demonstrated that if sufficient interval is maintained between two injections of streptomycin, the cats recovered from apnoea and the fall in blood pressure was not observed. This observation has been confirmed by the present study.

The degradation products, streptidine and streptamine both caused a pressor response. The absence of the stimulant action on blood pressure in reserpine treated cats suggests that this action is mediated through the release of catecholamines. These two compounds (10 and 20 mg/kg) also induced an insidious fall in blood pressure when injected every 10 min. The insidious fall was not present when they were injected every 30 min. In the case of the degradation products too, the neuromuscular blocking (Brazil et al. 1961) action could explain the hypotension observed on their repeated injection.

The antibiotics and the degradation products exhibited differences in their effects on the blood pressure. The degradation products had a stimulant action while the antibiotics had depressant actions. The addition of sugar residues to the respective

degradation products as in the case of streptomycin, kanamycin, neomycin and paromomycin and amino acid residues as in the case of viomycin not only masked the stimulant actions, but led to inhibitory effects.

The blood pressure response to vagal stimulation following the antibiotics and the degradation products was modified in all the experiments. The compounds inhibited the fall in blood pressure and expedited vagal escape. That the effect of vagus stimulation on the blood pressure was not mediated through an inhibition of nerve conduction was indicated by the absence of effects of the compounds on the contractions of the nictitating membrane in response to stimulation of the superior cervical ganglion.

Vagal escape has been shown to be effected by the release of large amount of acetylcholine which in turn leads to the release of adrenaline (Campose and Fredman 1963; Moore 1967). In cats, Miller et al. (1968) could not get any sympathetic vagal action by atropine treatment; however, they have not ruled out the release of "sympathin" to effect vagal escape.

An explanation for the vagal escape induced

by the antibiotics and the degradation products may be that they increase the release of acetylcholine which in turn leads to release of catecholamines culminating into vagal escape. Alternately the release of adrenaline and/or noradrenaline could be directly facilitated. The responses to electrical stimulation of the superior cervical ganglion of the cat were not potentiated by the antibiotics, while the potentiation following the administration of the degradation products though significant was not comparable to the potentiated blood pressure responses to McN-A-343, DMPP and histamine. This suggests that these compounds did not affect the release of the acetylcholine. The probability of a direct effect leading to the release of catecholamines, thus appears quite strong.

The vasodepressor response to histamine following the injection of antibiotics and the degradation products was followed by a secondary rise in blood pressure. This effect occured at the end of 60 minutes from the last injection of the cumulative dose and was present upto 3 hr. During this period the vasodepressor response to histamine was not affected ($P > 0.1$).

About 3 hr from the last injection of the cumulative dose of the antibiotics or the degradation products, the response to histamine was biphasic and the pressor component of the biphasic response to McN-A-343 was significantly increased. The pressor response to DMPP was also significantly increased and the vagal escape was facilitated. The blood pressure responses to adrenaline and noradrenaline were not affected and the vasodepressor component of the response to McN-A-343 was also not affected. The response of the nictitating membrane to stimulation of the superior cervical ganglion was also not significantly different from the control response. These results indicate an increase in the release of adrenaline and/or noradrenaline.

A secondary rise in blood pressure with histamine (10-15 µg) in spinal and decerebrate cats due to the release of adrenaline and noradrenaline was first reported by Burn and Dale (1926). That the release was caused by the adrenal glands and the peripheral stores was further confirmed by Szeygieslic (1932) and Bein and Meier (1953). The effect of histamine on adrenal glands of cats was also reported by Feldberg (1941) and Vogt (1951) who pointed out that the major portion of the

release from cats' adrenal glands consisted of adrenaline.

The intravenous dose of histamine required to release adrenaline and noradrenaline and to cause secondary rise as reported in the literature (Burn and Dale 1926, Slater and Dressel 1952 and Bein and Meier 1953) ranged between 10-20 µg. Thus a secondary rise in blood pressure to 0.1 µg/kg or 0.3 µg/cat (3 kg) of histamine following the antibiotics and their degradation products was considered to be of high significance. This effect of these antibiotics and their degradation products could be attributed to different mechanisms which are discussed below :

1. Sensitisation of the cardiovascular system to pressor substances : Sensitisation of the cardiovascular system of the cat to pressor substances like adrenaline and noradrenaline could be one possibility. Thus, though the quantal release of adrenaline and/or noradrenaline would remain constant, the responses would be potentiated because of sensitisation. The experimental results of blood pressure experiments however contradict such an assumption since the responses to adrenaline and noradrenaline following the administration of

antibiotics and their degradation products were not potentiated ($P > 0.1$). Moreover, the antibiotics and the degradation products did not potentiate the responses to adrenaline and noradrenaline of the rabbit aortic strip and the perfused rabbit ear artery. This indicates that the compounds do not sensitise the vascular system to adrenaline and noradrenaline.

2. Potentiation through block of up-take mechanism or Monoamine oxidase inhibition: Block of the uptake mechanism for noradrenaline or inhibition of monoamine oxidase could also lead to pressor response to histamine and increased pressor response to DMPP and McN-A-343. This possibility could however, be easily discounted since pressor responses to exogenously administered catecholamines were not potentiated.

3. Increased release of acetylcholine : The release of acetylcholine could be increased by the antibiotics and the degradation products and this could in turn cause greater release of adrenaline and/or noradrenaline. The response of nictitating membrane to stimulation of superior cervical ganglion following the antibiotics was not significantly affected. This excludes the possibility

of increased release of acetylcholine induced by the antibiotics.

The response of nictitating membrane to nerve stimulation following streptidine and streptamine though significant ($P < 0.05$) was not comparable with the potentiation of the blood pressure responses to McN-A-343 ($P < 0.01$) and DMPP($P < 0.01$) that followed administration of these compounds.

4. Increased release of adrenaline and/or noradrenaline : After the administration of the antibiotics and the degradationproducts, the state of stored adrenaline and/or noradrenaline could be so altered that these two amines are maintained in a highly labile condition. The labile condition of the transmitters could cause pronounced release or spilling over by the stimuli.

The results confirm such a hypothesis as the secondary rise in blood pressure in response to histamine was observed in all the cats, and the pressor response to McN-A-343 and DMPP was also increased. The fall in blood pressure on vagal stimulation was inhibited and vagal escape was facilitated. All these observations suggest that the mechanism for the release of the catecholamines

was modified. This holds true for both the antibiotics and their degradation products. The likelihood of the adrenaline or the acetylcholine-like actions of streptidine and streptamine playing a role in this phenomenon cannot be ruled out. Obviously, the sugar residues and the amino acid residues do not seem to interfere in this action of streptidine and streptamine.

The release of adrenaline and/or noradrenaline in response to histamine has been investigated under diverse conditions. Thus, Slater and Dressel (1952) reported secondary rise in blood pressure in response to histamine following competitive ganglion blocking agents like tatraethylammonium and hexamethonium. Trendelenberg (1961) has reported potentiation of secondary rise in blood pressure with histamine in spinal cats during competitive blocking phase of nicotine. Hexamethonium blocked the potentiation obtained during the competitive phase of nicotine and the response returned to its control value. Hexamethonium did not affect the pressor response to histamine obtained in spinal cats. The pressor responses to McN-A-343 and AHR-602 were similarly potentiated during the competitive blocking phase of nicotine. Barczak and Vane

(1965) have reported that the ganglion blocking agents like pentolinium, hexamethonium and mecamylamine increase the release of adrenaline and noradrenaline in response to histamine.

It is evident that competitive ganglion blocking agents affect the storage of adrenaline and/or noradrenaline so that the release in response to histamine, pilocarpine, McN-A-343 and DMPP is increased. The fact that a similar effect of histamine during the competitive blocking phase of nicotine was also observed (Trendlenberg 1961) strengthens the above deduction.

Neomycin (Ramos et al. 1961), kanamycin (Corrado and Ramos, 1960) and viomycin (Brazil et al. 1961) have been reported to possess ganglion blocking actions. Corrado (1958) has reported that streptomycin changed the vasodepressor response to pilocarpine into a biphasic response. This modification of the response to pilocarpine was attributed by the author to the ganglioplegic activity of streptomycin. Corrado (1958) has also reported that streptomycin inhibited the fall in blood pressure in response to stimulation of the vagus. The salivary secretion which was reduced following the injection of streptomycin was reinstituted by

the administration of neostigmine, and the rate of salivary secretion after neostigmine was pronounced and greater than that observed prior to the administration of streptomycin. These effects were also attributed to a ganglioplegic action of streptomycin.

In conclusion, it is suggested that the release of adrenaline and/or noradrenaline is facilitated by the antibiotics and the degradation products namely, streptidine and streptamine. That this release is not due to the increased liberation of acetylcholine is evident by the fact that the response of the nictitating membrane to stimulation of the superior cervical ganglion was not affected. Some other mechanism is probably involved in the facilitation of the release of adrenaline and/or noradrenaline.