DISCUSSION

For studying drug actions on the ganglion, the superior cervical ganglion of the cat has been extensively used because the ganglion cells are anatomically identical. In the present study, the effects of intra-arterially administered ganglion blocking agents, hexamethonium, tetraethylammonium, mecamylamine, pempidine, chlorisondamine and pentolinium were analysed on the dog superior cervical ganglion. The contractile responses of the nictitating membrane to preganglionic nerve stimulation were antagonised by all the blockers. The frequency-response curves were shifted to the right in a parallel fashion by hexamethonium and tetraethylammonium indicating that these agents blocked the dog superior cervical ganglion. The pA_2, pA_{10}, $pA_2 - pA_{10}$, and slope values were computed from the regression line according to the method of Arunlakshana & Schild (1959). This involved the assumption that the doses administered intra-arterially were distributed to the ganglion. The slope values of the regression lines of pA plots for hexamethonium and tetraethylammonium were not significantly different from the theoretical value of unity for competitive antagonism. Mecamylamine, pempidine, chlorisondamine and pentolinium produced parallel shifts of the frequency-response curves with lower doses but with higher doses the shift was not parallel and there was progressive flattening of the curves with reduction of maximal responses. Thus the antagonism with mecamylamine, pempidine, chlorisondamine and pentolinium was competitive only with lower doses; with higher doses the antagonism was not competitive. This conclusion supports the findings of Bennet et al, (1957); van Rossum & Ariens (1959); Ariens (1964) and Spink et al, (1958). The order of potency (pA_2 value) at the superior

cervical ganglion was hexamethonium > chlorisondamine ≫ mecamylamine ≫ pempidine > pentolinium > tetraethylammonium. Obviously, hexamethonium was the most potent blocker of the superior cervical ganglion of the dog. This is intriguing, since all previous studies (van Rossum & Ariens, 1959; van Rossum, 1961, 1962 a, b;) and the present one with the other tissue preparations are strongly indicative of the higher potency of chlorisondamine, mecamylamine, pempidine and pentolinium. A possible explanation may be that hexamethonium was injected directly into the blood supply of the ganglion and, therefore, the problem of diffusion barrier was reduced to minium.

Feldberg (1951) suggested the presence of ganglia in the guinea pig ileum. Trendelenburg (1961 a) suggested that the observations on the smooth muscle with the ganglion stimulants are made on structures which are not strictly comparable to the ganglion cells of the nervous system. A study of the present type on the ileum is not devoid of objections such as (i) the possibility that the ganglion blocking substances may have an atropine-like activity i.e. they block acetylcholine released from the postganglionic fibres close to the receptors of the smooth muscle; (ii) the ganglion blocking property of high concentrations of nicotine-like ganglion stimulants; (iii) the ganglion stimulating substance itself has a direct muscarinic action on the smooth muscle in addition to its effects on the intramural ganglion cells. Day & Vane (1963) made a parallel study of the effects of the

DMPP and acetylcholine and concluded that DMPP acted mainly at neural sites in the ileum. In the present study with the ileum preparations hexamethonium, tetraethylammonium and lower doses of mecamylamine, pempidine, chlorisondamine and pentolinium caused parallel shifts of the dose-response curves of nicotine and DMPP. Higher doses of the latter four blockers did not produce parallel shifts of the dose-response curves and there was a progressive flattening of the curves with reduction of the maximal responses. Therefore, it can be concluded that hexamethonium and tetraethylammonium exhibited competitive antagonism against nicotine and DMPP and this was further substantiated by the slope values of the regression lines close to unity. On the other hand, mecamylamine, pempidine, chlorisondamine and pentolinium did not exhibit competitive antagonism against the two agonists and this was supported by the slope values of the regression lines significantly different from unity. These findings confirm the observations on the dog nictitating membrane made in the present study and those of the earlier workers (Bennet et al, 1957; van Rossum & Ariens, 1959; van Rossum, 1961; Spink et al, 1958). Ambache & Lessin (1955) demonstrated that acetylcholine action remained unaltered while that of DMPP was abolished after the treatment of the gut with botulinum toxin. These reports notwithstanding, there is evidence, that DMPP at higher doses acts directly (Day & Vane, 1963; Soncin & Maffii, 1959). van Rossum (1961, 1962 a,b) used a pyridine-3-methyl-trimethylammonium (PMTM), nicotine and DMPP to study the nature of antagonism of the ganglion blockers viz.pentamethonium, hexamethonium, azamethonium, trimetaphane (ARFONAD), tetraethylammonium, chlorisondamine,

pentacyne (PRESIDAL), mecamylamine and pempidine and showed that DMPP and nicotine are partial agonists. PMTM was found to use receptors other than nicotinic. Therefore, in the present study, PMTM was not used and an attempt was made to see, if there was any difference in the antagonism exhibited by these compounds against nicotine and DMPP. Interference by the blocking effects of nicotine and DMPP at the higher doses was overcome by loading the recording lever lightly and by adjusting their doses in such a way that only four or five points lying on the ascending limb of the dose-response curves were selected. When complete dose-response curves were elicited they were "bell" shaped or there was autoinhibition.

As stated earlier, hexamethonium and tetraethylammonium acted competitively against nicotine and DMPP. These findings were further supported by the experiments made to get the recovery of the responses to DMPP after hexamethonium and chlorisondamine. The time taken (75 min) for recovery after chlorisondamine was greater than the time taken after hexamethonium (15 min). In general, it was observed with rabbit and guinea pig isolated ileum that the recovery of the responses occured after 50 to 70 min following high doses of mecamylamine, pempidine, chlorisondamine and pentolinium and after 15 to 20 min following high doses of hexamethonium and tetraethylammonium. These findings were further substantiated by the Paton & Rang (1965) analysis according to which when two antagonists compete to occupy the same receptors then the dose ratio (DR) = $(DR_1 + DR_2 - 1)$ and when these antagonists occupy different receptors, DR becomes the product of the dose ratios of the two antagonists given separately $(DR_1 \times DR_2)$ where DR_1 and DR_2

are the dose ratios of each of the antagonists given separately. The dose ratios were 9 and 4 respectively when hexamethonium and chlorisondamine were given separately and when they were given together the dose ratio was 38 which is close to 36, the product of the individual dose ratios. Results with cummulative and single exposures of the tissue to the same dose of hexamethonium and chlorisondamine also strengthen the above conclusion. The dose ratio with cummulative exposure to a given dose of hexamethonium was not different from that with single exposure to the same dose given all at once. However, with chlorisondamine cummulative exposures yielded higher dose ratios than single exposure to the same dose. The duration of action of hexamethonium was shorter than that of chlorisondamine supporting the earlier conclusion that the former acted competitively while the latter did not. The results obtained by the use of hexamethonium and chlorisondamine in combination against DMPP also suggested that hexamethonium afforded protection to the receptors against the effect of low doses of chlorisondamine. However, against higher doses of chlorisondamine protection was not afforded by hexamethonium. These findings confirm the results of McIsaac & Millekschoen (1963) who showed that hexamethonium in combination with chlorisondamine increased both the duration and intensity of the block and suggested that this was due to the occupation of large number of receptors when the two drugs were used in combination.

Atropine acts at the postganglionic parasympathetic effectors and blocks acetylcholine competitively (Arunlakshana & Schild, 1959). In the present study, the blocking action of atropine of the responses of the isolated rabbit ileum to DMPP was not competitive since there was a nonparallel shift to the right of the dose-response curves and the slope values of regression lines of pA plots were significantly different from unity. These findings are in agreement with those of van Rossum (1962 a,b) who reported a nonparallel shift of the dose-response curves of nicotine by atropine with guinea pig jejunum.

When responses of the rabbit ileum to nicotine and DMPP were registered with auxotonic lever the phenomenon of "fade" reported by Paton (1959, 1961) was not observed for 3 to 4 hr. As far as the maximum in the time-response curve and, therefore, the "fade" phenomenon is concerned, the use of an isometric or auxotonic lever for registering the responses (Paton, 1959, 1961) may easily induce such a phenomenon as an artifact. In the isolated organs brought to contraction under isometric conditions, high tensions are built up to which the organ may give way by a change in shape, i.e. elongate. This in turn will result in a "fade" in the tension developed originally. Even with the isotonic registration, the further the muscle contracts, the more the originally isotonic contractions become isometric. This implies that the internal tensions built up in the piece of the gut may possibly lead to the "fade" phenomenon. The "fade" can easily occur as an artifact. For the experimental study of the time response relations isotonic registering seems to be safest. The possible reason for not observing the "fade"

phenomenon in rabbit isolated ileum (in the present study) could be due to the presence of strong rhythmic pendular movements which may act as check to the high tension built up.

The results with guinea pig ileum were similar to the findings with the rabbit ileum i.e. hexamethonium and tetraethylammonium antagonised nicotine and DMPP competitively whereas antagonism by mecamylamine, pempidine, chlorisondamine and pentolinium was not competitive. "Fade" discussed above and reported by Paton (1959, 1961) developed very soon when responses were recorded with auxotonic lever. Therefore, a detailed study could not be done with this lever. These observations confirm the findings of Paton (1959, 1961) in connection with the development of the phenomenon of "fade" and also of Ariens (1964) who reported the development of "fade" in the heavily loaded guinea pig ileum and its absence in the lightly loaded piece of guinea pig ileum. The appearance of "fade" in guinea pig ileum may be due to lack of strong pendular movements. The curves obtained with DMPP or nicotine with guinea pig ileum were more steep in the absence of the antagonists confirming the findings of Trendelenburg (1961 b).

The guinea pig hypogastric nerve vas deferens was used to study the action of ganglion blockers on the peripheral ganglia situated on the hypogastric nerve. The existence of the ganglia on this preparation was under great controversy in the early sixties. Chang & Rand (1960) and Burnstock & Holman (1961,1962) assumed that the majority of fibres in the hypogastric nerve distal to the inferior mesentric ganglion were postganglionic. Sjostrand (1962)

however, provided clear indication of the existence of synaptic relay in the hypogastric nerve, and histological studies by Merrillees et al, (1963) have demonstrated ganglionic cells in this nerve close to its entry into the vas deferens. Bentley (1962), reported that bretylium, guanethidine, nicotine and DMPP at high doses blocked the responses to hypogastric nerve stimulation while physostigmine and carbaminoyl choline potentiated the responses. Birmingham & Wilson (1963) suggested the presence of ganglia on the hypogastric nerve because transmural stimulation of the vas deferens produced contractions which persisted in the presence of high concentrations of hexamethonium, pentolinium, nicotine, DMPP, and mecamylamine which simultaneously abolished the responses to hypogastric nerve stimulation. The adrenergic nature of the postganglionic fibres to the vas was demonstrated by the use of dihydroergotamine and phentolamine, which abolished the responses to postganglionic nerve stimulation and to added noradrenaline. Bretylium and guanethidine also blocked the responses to nerve stimulation at the adrenergic neurone blocking dosage. Bentley & Sabine (1963) also did similar type of studies for showing the presence of ganglia on the hypogastric nerve supplying the vas deferens. Hexamethonium, nicotine, pempidine, and d-tubocurarine had no blocking action on the responses to transmural stimuli, yet at the same concentrations they completely blocked the contractions due to stimulation of the hypogastric nerve. The authors claimed, that these drugs have no postganglionic sympathetic blocking action, but act solely on the ganglia in the hypogastric nerve. Hemicholinium produced a complete block of the responses to stimulation of the hypogastric nerve, whereas the transmural stimulation was

partially blocked. On the other hand, hemicholinium did not reduce the responses to added noradrenaline. Thus, these authors proposed that hemicholinium, like hexamethonium produced most of its blocking action on the hypogastric nerve by blocking the ganglia, not by an action on the adrenergic neurones. Ohlin & Stromblad (1963) using this preparation with the nerve electrodes moved to a point 1-5 mm away from the organ produced contractions which resisted ganglionic-blocking concentrations of hexamethonium. By staining the hypogastric nerve with methylene blue, these authors showed the presence of ganglionic cells near the vas deferens. These cells flouresced when examined by the method of Falck (1962) for monoamine.

In the present study, resistence of the responses to block by hexamethonium, tetraethylammonium, mecamylamine, pempidine, chlorisondamine and pentolinium was observed when the stimulating electrodes were placed at 1-5 mm away from the organ (the electrodes used were the protected and sliding types whereby there is very little chance of stimulating the vas deferens directly). However, the ganglion blockers blocked the responses to stimulation of preganglionic nerve trunk. The order of potency, in general, was the same as obtained with the isolated rabbit and guinea pig ileum i.e. chlorisondamine was the most potent followed by mecamylamine, pempidine, pentolinium, hexamethonium and tetraethylammonium in that order. All the blockers shifted the frequency response curves to the right; with lower doses the shift was parallel, whereas it was nonparallel with higher doses and there was progressive flattening of the curve and reduction of the maximal response. These findings indicate that at lower doses the

antagonism was competitive and at higher doses the antagonism was not competitive. Hence, it could be concluded that none of the blockers tested acted competitively in the enlisted range of doses at the peripheral ganglion situated on the hypogastric nerve. Perhaps, there was a combination of competitive and "not competitive" antagonism. This difference in the nature of antagonism could be explained on the basis of behaviour of the ganglia reported by Zaimis (1965), who showed that after treating the rats with nerve growth factor antisera for producing immunosympathectomy, the mesenteric ganglion escaped destruction whereas paravertebral ganglion was completely destroyed and the coeliac ganglion was atrophied to the extent of 75%. Vas deferens after this treatment showed an increase in the uptake of perfused H^3-noradrenaline whereas heart, spleen, lungs, kidney, small intestine and colon showed a reduction in the uptake of the labelled noradrenaline. Another interesting instance of antiserum resistance was observed in the peripherally located adrenergic ganglia which provide for the innervation of the vas deferens or the uterus. A histochemical investigation by Hamberger et al, (1965) showed the persistence of the flourescent adrenergic nerve fibres around the vas deferens of the rats treated with anti-serum. The site of origin of these fibres was traced to a large ganglionic complex in close apposition to the caudal portion of the vas deferens. This ganglion was only slightly reduced in size in the experimental animals and the individual neurones were apparently normal. Hamberger et al, (1965) concluded that these adrenergic neurones are structurally and functionally different from other adrenergic neurones. Such findings they pointed out, "call for caution" in the

interpretation of pharmacological data with this test organ. "Dense-core" granular vesicles are present in large number in controls whereas they occur extremely rarely, if at all, in the heart of rat treated with the anti-serum. The same vesicles were reported to be entirely absent from the submaxillary fraction of immunosympathectomized mice and rats but were intact in the vas deferens (Sjoqvist et al, 1965).

The existence of unusual ganglionic stimulatory pathways has been pointed out right from forties. Salerno & Coon (1949); Holmstedt (1951); Root (1951) and Long & Eckstein (1961) reported that the intravenous administration of pilocarpine, neostigmine and other cholinesterase inhibiting agents in a hexamethonium or tetraethylammonium treated dog elicited a pressor effect instead of a depressor effect and this was blocked by atropine. Roszkowski (1961) reported that the pressor activity of McN-A-343 is due to stimulation of sympathetic ganglion and adrenal medulla. Further, intra-arterial administration of McN-A-343 into the superior cervical ganglion elicited stimulation of nictitating membrane and intravenous administration of the same dose failed to stimulate the nictitating membrane and injections to the membrane failed to show the effect. Section of the postganglionic fibre of the superior cervical ganglion did abolish the effect. The action of McN-A-343 at the superior cervical ganglia was similar to that at the other sympathetic

ganglia as responses were abolished in sympathetctomized and adrenalectomized animals. The same authors proposed that McN-A-343 and atropine act specifically at certain receptor areas in a mutually antagonistic and competitive manner. That this antagonism occured at ganglionic sites was indicated by experiments in which it was shown that the contraction of the nictitating membrane resulting from ganglionic stimulation induced by direct intra-arterial administration of McN-A-343 to the superior cervical ganglion was selectively antagonised by injection of atropine in the same area. Other workers have also reported blockade by atropine at the ganglion. Trendelenburg (1954) showed atropine antagonism against pilocarpine. Franko et al, (1963) reported similar pharmacological properties of another synthetic agent, N-benzyl-3-pyrrolidyl acetate methobromide (AHR-302). Bainbridge & Brown (1960) reported that atropine and other peripheral parasympathetic blocking agents are not appreciably less potent than ganglionic blocking agents i.e. hexamethonium in producing ganglionic block due to preganglionic nerve stimulation. Atropine exhibited a longer duration of block against McN-A-343 than against the nerve stimulation. Levy & Ahlquist (1962) emphasized the similarities between groups of the ganglion stimulants that are antagonised by conventional blockers and those that are sensitive to atropine inhibition.

The present study shows that atropine acted competitively since it caused a parallel shift of the agonist dose-response curves and when the data were subjected to analysis according to the method

of Arunlakshana & Schild (1959), the hypothesis of competitive antagonism was confirmed.

It is concluded that hexamethonium and tetraethylammonium acted as competitive antagonists at the superior cervical ganglion of dog, and rabbit and guinea pig ileum. Hexamethonium, tetraethylammonium, mecamylamine, pempidine, chlorisondamine, and pentolinium did not act competitively at the peripheral ganglia on hypogastric nerve innervating vas deferens. Mecamylamine, pempidine, chlorisondamine, and pentolinium did not act competitively at the superior cervical ganglion of dog and rabbit and guinea pig ileum. Atropine antagonised competitively the responses to the two "non-nicotinic" ganglionic stimulants, muscarine and McN-A-343 at the superior cervical ganglion of the cat.