CHAPTER IV

DISCUSSION
The various experimental procedures and observations have been described in detail in the foregoing chapters. Attempts will now be made to provide possible interrelationships of the effects observed with a view to elucidating the mechanism of action of the drug under study. It may be mentioned that direct evidence for such interpretations was not always available and in some cases speculative explanations have been offered on the basis of available reports in the literature.

Oxotremorine on administration to anaesthetised rat produced hypotension and bradycardia. This cardiovascular effect of oxotremorine was found to be abolished in animals pretreated with atropine methyl nitrate which does not pass the blood-brain barrier (Innes & Nickerson, 1971). However pretreatment with ganglion blocking drug failed to alter the hypotensive effect (Table 1) indicating peripheral sites of action in respect of this hypotensive effect of oxotremorine. Antihistaminics e.g. mepyramine also were not found to be effective in abolishing the vaso-depressor effect of oxotremorine (Table 1). This failure of ganglion blocking and antihistaminic agents to abolish the hypotensive effect and antagonism
of this effect by a peripherally acting cholinolytic
drug together suggest that the hypotensive effect of
oxotremorine is peripheral muscarinic in nature. These
findings therefore support previously reported observa­
tions (Cho et al., 1962; Levy & Michel-Ber, 1965).

The hypotensive response to oxotremorine is
abolished in rats pretreated with atropine revealing
on the other hand a prolonged hypertensive effect on
administration of high dose of oxotremorine(Table 2,
Fig 1a). Oxotremorine is found to be as potent as ace­
tylcholine so far as the muscarinic property is concerned
(Cho et al, 1962). But oxotremorine was not found to
share the nicotinic effect of acetylcholine (Levy &
Michel-Ber, 1967; Cho et al, 1962). Therefore the hyper­
tensive effect in atropinised rat as observed in these
experiments is regarded as a novel pharmacological effect
not reported so far.

Similar muscarinic agents capable of causing tremor
like physostigmine (Lesic & Varagic, 1961; Varagic,1955
and Varagic & Vojvodic, 1962) and arecoline (Hunt &
Renshaw, 1929) do exhibit hypertensive effect in experi­
mental animals. Thus it seems to be more important to
give particular emphasis to the examination and critically
analysis of this effect of oxotremorine and its interre­
lationship with the tremorigenic property of oxotremorine.
It was difficult to anticipate the mechanism of the observed vasopressor effect. Considering the immediate and rapid onset of the effect an indirect mechanism did not appear to be involved in this response. On the contrary, the reported peripheral effect of oxotremorine on cardiovascular and other systems failed to explain the mechanism of this response. An interesting observation of De-Groat and Volle (1963) on a ganglio-excitatory effect of oxotremorine may have some bearing on this response. Moreover a purely peripheral sympathetic overactivation (Fig 7, Table 3) has also been demonstrated of the responses of vas deferens to sympathetic nerve stimulation. These observations were kept in view while examining the mechanism of the aforesaid effects of oxotremorine.

The participation of α-adrenoreceptor in the mechanism of action of pressor response to oxotremorine was evidenced in the present experiments as dibenzyline, an α-adrenolytic agent could effectively block the vasopressor effect (Fig 3, Table 2). This observation therefore directly pointed to either nor-adrenaline/adrenaline or both being involved in the pressor effect of oxotremorine. Whether this effect was due to the direct activation of adrenergic receptor or due to an indirect mechanism could not be ascertained at this stage. Further clarification of the exact nature of sympathetic involvement will
be discussed shortly. Studies on interaction between bretylium (Boura & Green, 1959) or guanethidine (Hertting et al., 1962), two adrenergic neurone blocking agents and oxotremorine excluded the possibility of direct activation of adrenergic receptors. It was observed that vasopressor effects of oxotremorine was either inhibited or markedly reduced when readministered after injection of bretylium and guanethidine (Fig 3, Table 2). This observations indicated that possible sites of vasopressor responses to oxotremorine after cholinergic blockade could be either at the level of sympathetic neurone or above. Similar experiments were performed to examine the influence of ganglionic blocking agents on the vasopressor effects of oxotremorine. The pressor responses to oxotremorine as had been observed was prevented on readministration after ganglionic blockade. But complete abolition of the pressor effects was rarely observed after ganglionic blockade (Fig 3, Table 2). The extent of pressor effects which existed even after ganglionic blockade was found to be $33.4 \pm 3.72 (S.E)\%$ in a total of 6 experiments. The pressor response which still remains after ganglionic blockade could be mediated through adrenergic neurone, the possibility of which was later examined on intramurally stimulated vas deferens preparation. The marked prevention of the pressor effect after ganglionic
Block effect suggested the locus of action to be either at the ganglia or at the central nervous system. To elucidate the role of adrenal medulla in the pressor response to oxotremorine the effects of oxotremorine was examined on adrenalectomised rat. Prevention of vasopressor response to oxotremorine in adrenalectomised rat (Fig 1c, Table 2) suggested participation of adrenal gland presumably by releasing adrenaline or noradrenaline under the influence of oxotremorine. To ascertain whether this hypertensive effect was mediated through a central mechanism the vasopressor effect of oxotremorine was evaluated in pithed rats. It was evidenced that by destruction of brain and spinal cord, the vasopressor response to oxotremorine after intravenous injection in atropinised rat could be completely prevented (Fig 1d). The series of experiments carried out so far revealed that oxotremorine-induced pressor effect was mediated through a central mechanism bringing about liberation of nor-adrenaline from the adrenal medulla. To finally confirm the liberation of nor-adrenaline by oxotremorine, its effects were examined in reserpinised rats where store of tissue catecholamines were completely depleted. In accordance with the observation discussed in the foregoing pages the vasopressor response to oxotremorine was found to be diminished to a great extent in such experiments (Fig 1b, 3, Table 2).
The role of indirect mechanism in the effect of oxotremorine was further strengthened by the observation that repeated administration of the same dose of oxotremorine resulted in gradual decrease in extent of the effect, finally showing complete tachyphylaxis (Fig 2). This observation fitted with our hypothesis of nor-adrenaline depletory effect of oxotremorine. Attempts were made at this stage to restore the vasopressor effect by infusion with nor-adrenaline after establishment of complete tachyphylaxis. Such treatment could reverse partially the vasopressor effect to a maximum extent of 10.9% (of control blood pressor response to 1st dose) (Fig 2). The interpretations offered for the vasopressor effects of oxotremorine as discussed earlier finally lead to the conclusion that vasopressor response to oxotremorine in atropinised rat was due to centrally mediated release of nor-adrenaline primarily from adrenal medulla. Release of nor-adrenaline, from other peripheral sites is also possible, but of lesser importance, which was evidenced in subsequent experiments with isolated tissues.

The centrally mediated sympathetic overactivation has been observed by Gyorgy et al. (1971). Oxotremorine-induced central stimulatory effects have been observed in a variety of species viz. chickens, pigeons, mice, rats, guineapigs, rabbits, cats, dogs and monkeys when
the animals were pretreated with methyl atropine to eliminate the peripheral effects (George et al., 1962). Cats and monkeys showed symptoms of pacing, jumping, and circling followed by a ragelike syndrome. The reported observation of Walker and Weetman (1970) who have noted hypertensive effects of oxotremorine in methyl atropine-treated animals and its antagonism by administration of atropine sulphate stand distinctly against the result obtained from experiments described here. Walker and Weetman (1970) have finally concluded that oxotremorine-induced pressor response is due to stimulation of central cholinoreceptors. At this juncture it was felt necessary to critically analyse the observations on the effects of oxotremorine after atropine and its quaternary derivatives to finally determine the muscarinic or adrenergic participation in pressor response to oxotremorine. In the earlier part of our study atropine sulfate was injected intramuscularly in all the animals. The intramuscular route was chosen because intravenous injection sometime caused an acute drop of basal blood pressure and interfered with the experiments. In course of our critical comparison of the pressor responses to oxotremorine between atropine and methyl atropine-treated animals, both the drugs were injected through the intravenous route very carefully and at the dose level employed by Walker and Weetman (1970).
As a result of comparative studies on the vaso­pressor responses to oxotremorine in atropine and methyl atropine-treated animals the following three important observations were made which are distinctly different from most of Walker and Weetman (1970).

1. The oxotremorine induced vasopressor response in atropinised animal (Fig 1a).
2. Abolition of pressor responses to oxotremorine in adrenalectomised animal (Fig 1c).
3. Development of tachyphylaxis to pressor responses of oxotremorine (Fig 2).

These pertinent observations on the pressor responses to oxotremorine are not entirely supportive of the conclusion that the mechanism of oxotremorine-induced hypertensive effect is due to participation of central muscarinic receptors.

Both central and peripheral muscarinic receptors are blocked by atropine. Therefore hypertensive response by oxotremorine in atropine-treated animal exclude the possibility of involvement of central muscarinic receptors in the pressor response to oxotremorine. The hypothesis of liberation of noradrenaline by oxotremorine to bring about the pressor response is supported by the observed abolition of this effect by surgical adrenalectomy. Friedman and Campos (1960) have also observed the centrally mediated release of catecholamines from adrenal medulla by
oxotremorine. The development of tachyphylaxis to the pressor response of oxotremorine suggested the involvement of an indirect mechanism. It has been observed during the course of studies on the development of tachyphylaxis to pressor effect of oxotremorine that the pressor effect became tachyphylactic at the 5th dose in atropine-treated animals, on the other hand in methyl atropine treated animal this response became tachyphylactic at the 8th dose. Therefore, the delay in development of tachyphylaxis to the pressor response in methyl atropine-treated animal is difficult to explain. The extent of blockade of cholinceptors in the atropine-treated animals, where both central and peripheral muscarinic effect are blocked, is more than in methyl atropine-treated animals where only peripheral muscarinic receptors are blocked. Therefore the degree of alteration in the cholinergic - adrenergic balance will be greater in atropine-treated animal than in the methyl atropine-treated animals. Possibly it was due to this difference in the loss of balance in cholinergic-adrenergic system that the difference in the development of tachyphylaxis was caused. The other possible explanation for rapid development of tachyphylaxis in atropine-treated animal may be the facilitatory effect of atropine on release of nor-adrenaline, as Muscholl (1970) observed that atropine caused facilitation of release of nor-adrenaline from peripheral sites.
Therefore the mechanism of vasopressor effects of oxotremorine in atropinised rats is primarily due to centrally mediated release of noradrenaline coupled with some insignificant release of catecholamine from the peripheral tissues. Although the results as discussed in the previous pages primarily indicated a central component for sympathetic predominance, it was necessary to exclude the possibility of any peripheral mechanisms in such action. This was particularly important since oxotremorine was found to produce some vasopressor effects even after ganglionic blockade and adrenalectomy. The use of guineapig hypogastric nerve-vas deferens and transmurally stimulated vas deferens preparations served as very convenient techniques for this purpose. These two preparations served as a representative miniature unit of the sympathetic nervous system permitting stimulation of preganglionic nerve or post ganglionic neurone (Bentley & Sabine, 1963; Birmingham & Wilson, 1963). The facilitation of responses to sympathetic stimulation by oxotremorine was found to be more marked in the case of hypogastric than in that of intramural stimulation (Table 3).

A possible explanation for the difference in the extent of oxotremorine effect could be the involvement of both the sites in sympathetic ganglia and neurone. Assuming this to be so, the extent of facilitation is expected to be more in the Hukovic preparation where both ganglia and neurone are
present than transmurally stimulated preparation in which the ganglia are absent. These results therefore confirm the ganglioexcitatory effect of oxotremorine as reported by other workers (Friedman & Smith, 1962; De-Groat & Volle, 1963).

Oxotremorine in these experiments was found to be devoid of any influence on the adrenergic receptors. The potentiatory effect of oxotremorine on the response to exogenous adrenaline which coincided with the sympathetic facilitation could be due to an additive effect of endogenous noradrenaline liberated by oxotremorine. The liberation of noradrenaline from the storage sites of tissue was evidenced as oxotremorine failed to produce any sympathetic facilitation in the reserpinised preparation (Fig 11, Table 3). Further confirmation of these hypotheses was provided by the development of partial tachyphylaxis of the facilitatory effect on repeated administration (Fig 9, 14). Similar observations indicating capacity of oxotremorine to release noradrenaline from the peripheral tissue have also been reported by Anton et al. (1967).

Thus it can be concluded from these observations that oxotremorine may cause signs of sympathetic activation through indirect release of transmitter, in addition to its centrally mediated effects as discussed earlier.
It is perhaps pertinent to mention that oxotremorine is capable of producing cholinergic effects by an indirect mechanism through increased release of acetylcholine (Gyorgy et al., 1970). Moreover participation of acetylcholine in adrenergic transmission has been well documented in the literature (Burn & Rand, 1959; Burn & Rand, 1961; Burn & Rand, 1962; Burn & Rand, 1965). The facts which have been discussed above indicate that oxotremorine induces a marked activation of the sympathetic system. The oxotremorine induced sympathetic overactivation is mediated both at central and peripheral sites. The observed central sympathetic overactivation by oxotremorine is the direct effect of the drug.

To examine the role of the cholinergic link in bringing out its facilitatory effect on sympathetic responses studies were undertaken on the interaction between oxotremorine and hemicholinium-3, a well known inhibitor of acetylcholine synthesis (MacIntosh et al., 1956). The blockade of the responses of vas deferens to nerve stimulation by hemicholinium-3(HC-3) has been construed as evidence of cholinergic mediation in the activity of sympathetic nerve (Chang & Rand, 1960).

The results indicated that prior incubation with HC-3 significantly antagonised the facilitatory effect of oxotremorine in the preparation(Fig 10,15). Thus possibility
of cholinergically mediated sympathetic facilitation cannot be ignored.

Besides affecting the autonomic nervous system oxotremorine has been reported to be active at skeletal neuro-muscular site (Elmqvist & McIsaac, 1967; Csillik, 1964; Ganguly & Chaudhuri, 1970). The importance of skeletal neuromuscular involvement is obvious since the skeletal muscle happens to be the ultimate target organ for production of the parkinsonimimetic effects like tremor and rigidity. That is precisely the reason for examining the effects of oxotremorine at myoneural junction.

The results obtained from the studies of myoneural effect of oxotremorine suggest that the neuromuscular blockade produced by oxotremorine is depolarising in nature. Oxotremorine produces tetany like fasciculations at low doses (0.5 to 1.0x10^-6 g/ml) and blockade at higher concentration (2.0 to 8.0x10^-6 g/ml). The neuromuscular blocking effect of oxotremorine was found to be potentiated in presence of physistigmine (Table 4). Elmqvist and McIsaac (1967) have suggested that depolarisation of muscle end plate is caused in skeletal myoneural junction after a very high dose of oxotremorine (5.0x10^-5 g/ml). The results presented in the study show that oxotremorine fails to produce any spasmogenic effect on frog rectus abdominis preparation. Besides, the responses of frog
rectus abdominis preparation to acetylcholine were not altered by oxotremorine. These observations are not supportive of direct depolarisation of the motor end plates by oxotremorine. Release of acetylcholine at skeletal myo-neural junction by tremorine is suggested by Csillik (1964). Ganguly and Chaudhuri (1970) have studied the effect of oxotremorine at skeletal neuromuscular junction and suggested that myoneural effect of oxotremorine is possibly mediated through release of acetylcholine.

The studies on the level of acetylcholine in peripheral tissues, show that oxotremorine causes a fall in the acetylcholine level of peripheral tissues (Table 6). This observation suggests that oxotremorine possibly causes release of acetylcholine in peripheral tissue and supports the suggestion put forward by Csillik (1964).

Release of acetylcholine in brain and smooth muscle is reported to be blocked by morphine (Paton, 1957; Cox & Wienstock, 1966). Recently Pinsky and Frederickson (1971) have reported that morphine blocks the release of acetylcholine at skeletal myoneural junction. The reduction in the degree of myoneural blocking effect of oxotremorine by morphine suggests that this effect of oxotremorine is directly dependent on the quantity of acetylcholine released at the myoneural junction. When
the synthesis of acetylcholine is inhibited by treatment with hemicholinium-3 (Macintosh et al., 1956) as evidenced by complete failure of transmission of nerve impulses, oxotremorine fails to manifest any paralytic effect on the twitch responses to direct stimulation. This observation shows that oxotremorine is devoid of any direct depolarising effect on the muscle end-plate.

As the neuromuscular effect of oxotremorine is blocked by drugs like morphine which reduces the release of acetylcholine and Hemicholinium-3 which reduces the availability of acetylcholine for release at myoneural junction by inhibiting the synthesis of acetylcholine, it may be fairly concluded that the mechanism of action of oxotremorine at myoneural junction is possibly through increased release of acetylcholine.

The peripheral parasympathomimetic effect of oxotremorine is well documented in the literature (Everett, 1956; Cho et al., 1962; Levy & Michel-Ber 1965). There are divergent views on the mechanism of action of the cholinomimetic effect of oxotremorine at the peripheral sites. The peripheral cholinergic effect of oxotremorine is thought to be of direct post-synaptic origin on the basis of the observation that the hypotensive effect of oxotremorine is not potentiated by pre-treatment with anti-cholinesterase (Cho et al., 1962). Levy and Michel-Ber (1967)
have observed that morphine which inhibits the release of acetylcholine (Paton, 1957) fails to reduce the spasmogenic effect of oxotremorine on guinea pig ileum. The observations of Levy and Michel-Ber (1967) are supportive of the direct effect of oxotremorine in respect of its peripheral parasympathomimetic effects.

Tremor induced by tremorine is antagonised by hemicholinium-3 in chick (Bowman & Osuide, 1968). Triethylcholine has also been shown to inhibit the tremorogenic property of tremorine in rat (Slater & Rogers, 1968). Therefore the antagonism of the tremorine-induced tremor by drugs like HC-3 (MacIntosh et al., 1956) and triethylcholine (Bull & Hemsworth, 1965) which inhibit the synthesis of acetylcholine is suggestive of an indirect mechanism of action of tremorine/oxotremorine. Gyorgy et al. (1970) have shown the antagonism of the spasmogenic effect of oxotremorine on rat urinary bladder by HC-3 and morphine. The spasmogenic effect of oxotremorine on rat urinary bladder has also been reported (Leszkovszky & Tardos, 1971) to be antagonised by 3,6 bis -(3-diethyl aminoproxy) - pyridazine - bis-methyliodide (WIN - 4981 -2) a hemicholinium like compound which inhibits the synthesis of acetylcholine (Levy & Ahlquist, 1962).
Therefore an attempt has been made to elucidate the mechanism of action of the peripheral parasympathomimetic effect of oxotremorine by employing various drugs which are known to affect the synthesis and release of acetylcholine. Preparations like guineapig ileum, rat colon, and rat urinary bladder and rabbit auricle which are predominantly sensitive to cholinergic drugs have been chosen for this purpose.

C₁₀Dichol is reported to be a potent inhibitor of the synthesis of acetylcholine (Barlow et al., 1953; Barlow, 1955; Barlow & Zoller, 1962, Hemsworth, 1971). The results of the experiments on guineapig ileum preparation show that C₁₀Dichol antagonises the spasmogenic effect of oxotremorine preferentially to that of acetylcholine.

C₁₀Dichol antagonised the oxotremorine and acetylcholine-induced contraction of guineapig ileum by 62.6 ± 1.88% and 41.5 ± 2.34 (S.E)% respectively (Fig 16, 17). HC-3, an inhibitor of the synthesis of acetylcholine (MacIntosh et al., 1956) also antagonised the spasmogenic effect of oxotremorine and acetylcholine by 59.8 ± 7.17 (S.E)% and 19.95 ± 5.65(S.E)% (Fig 18, 19). The preferential antagonism of the effect of oxotremorine by drugs which inhibit the synthesis of acetylcholine indicates the indirect mechanism. The antagonism of response
to acetylcholine by HC-3 on this preparation may be accounted for the atropine like property of HC-3 on the guineapig ileum (Bieger et al, 1968). The antagonism of the spasmogenic effect of acetylcholine by C\textsubscript{10}Dichol may possibly be due to its atropine-like effect on guineapig ileum preparation as observed in the case of HC-3 or non-specific spasmolytic effect.

However in rat colon preparations C\textsubscript{10}Dichol antagonises oxotremorine-induced contraction by $50.9 \pm 3.82$ (S.E)% but the response to acetylcholine remain unaffected (Fig 20,21). This observation suggests that antagonism of acetylcholine response by C\textsubscript{10}Dichol in guineapig ileum preparation may be due to the atropine like non-specific spasmolytic action of C\textsubscript{10}Dichol on this particular preparation. On rat colon preparation HC-3 antagonises the response to oxotremorine and acetylcholine by $47.19 \pm 5.25$ (S.E)% and $16.78 \pm 4.14$ (S.E)% respectively(Fig 22, 23). The antagonism of acetylcholine response by HC-3 may also be due to its atropine-like property (Bieger et al, 1968) on the preparation as observed in guineapig ileum preparation. Gyorgy et al. (1970) have reported that the atropine-like property of HC-3 is absent in rat urinary bladder preparation. The results presented here show that C\textsubscript{10}Dichol and HC-3 antagonised the oxotremorine induced responses whereas the acetylcholine induced responses
were potentiated (Fig 24, 25, 31, 32). These observations further confirm the report of Gyorgy et al. (1970) and provide evidence that C\textsubscript{10} Dichol fails to produce any atropine-like effect on rat urinary bladder, as observed in guineapig ileum preparations. The reversal of antagonism of oxotremorine induced contraction by C\textsubscript{10} Dichol and HC-3 when choline was pre-incubated in the bath fluid (Fig 27a, 28, 33) further confirm that the antagonism of the spasmogenic effect of antagonism by C\textsubscript{10} Dichol and HC-3 is due to inhibition of synthesis of acetylcholine, a property shared by both the drugs. The antagonism of spasmogenic responses to oxotremorine on rat urinary bladder by morphine (Fig 34) which is reported to cause inhibition of release of acetylcholine from smooth muscle (Paton, 1957) provides supportive evidence in favour of indirect mechanism in the peripheral muscarinic effect of oxotremorine.

On isolated rabbit atrium C\textsubscript{10} Dichol (0.16x10\textsuperscript{-6} g/ml) completely abolishes the negative ionotropic effect of oxotremorine but negative ionotropic effect of acetylcholine was not completely abolished (Fig 36, Table 5). The antagonism of the peripheral cholinomimetic effects of oxotremorine as studied in a variety of preparation, by drugs which either inhibit the synthesis or release of
acetylcholine convincingly suggest that the mechanism of peripheral parasympathomimetic effects of oxotremorine is an indirect one mediated through release of acetylcholine. A fall in the level of acetylcholine in peripheral tissues on administration of oxotremorine (Table 6) as observed in the biochemical experiments, also fits into this hypothesis.

The results presented here are in agreement with that of Gyorgy et al. (1970) who have also suggested an indirect mechanism, mediated through release of acetylcholine, in the peripheral parasympathomimetic effect of oxotremorine.

Observations have been reported on the causal relationship between changes in the level of brain acetylcholine and the tremor produced by tremorine and oxotremorine (Holmsted et al., 1963; Pepeu, 1963; Holmsted & Lundrangen, 1966). However Cox and Pötkonjak (1969a) have failed to correlate the rise of acetylcholine level in brain and tremorigenic property of drug. The antagonism of the tremorigenic effect of oxotremorine by HC-3 (Bowman & Osuidé, 1968) and triethylcholine (Slaters & Rogers, 1968) and the abolition of the peripheral cholinomimetic effect of oxotremorine by HC-3 (Gyorgy et al., 1970) and WIN-4981-2 (Leszkovszky & Tardos, 1971) indicate that the pharmacological effects of oxotremorine are mediated through an indirect mechanism in both central
and peripheral - cholinergic system. In the present investigation the effect of oxotremorine on the acetylcholine content of brain, heart and small intestine in rats have been studied with a view to establishing the relationship of changes in level of acetylcholine and pharmacological effects exhibited by oxotremorine. It is interesting to note that the increase in brain acetylcholine and decrease in acetylcholine content of peripheral tissues induced by oxotremorine was maximum at 10 min and subsided after 40 min. The maximum pharmacological effects (both tremor and autonomic effects) of oxotremorine are also manifested within 10 min and abolished within 1 hr. The fact that development and duration of pharmacological effects of oxotremorine correspond to the changes in the concentration of acetylcholine in the brain as well as in peripheral tissues suggests a probable role of acetylcholine in the mechanism of action of this drug. The antagonism of the peripheral autonomic effects e.g. smooth muscle stimulating property, negative inotropic effects on atrium and the neuromuscular paralytic effects of oxotremorine by \textit{C}_{10}\text{Dichol} and HC-3 which inhibit the synthesis of acetylcholine and morphine which blocks the release of acetylcholine and the fall in the level of acetylcholine in the peripheral tissue on administration of oxotremorine are
suggestive of an indirect mechanism of action of oxotremorine, mediated through release of acetylcholine from the peripheral sites.

The role of serotonin in relation to the mechanism of action of tremorine and oxotremorine is controversial. No change in the serotonin level of brain on administration of tremorine/oxotremorine was found by various workers (Everett, 1964; Cho, 1966). On the contrary, Whittaker and Walaszek (1964) and Jenden (1968) have demonstrated increase in the level of brain serotonin. Drug-induced tremors have been demonstrated to be potentiated on intraventricular administration of serotonin (Domer & Feldberg, 1960). A decrease in the urinary excretion of 5-hydroxy indole acetic acid (5-HIAA) a metabolite of serotonin (Barbeau & Jansmin, 1961) in Parkinson's patients indicate involvement of metabolic disorder of this amine in Parkinson's disease. The influence of tremorine/oxotremorine on serotonin content of peripheral tissues has not yet been reported.

The results of the present investigations show an increase of serotonin level in the intestine and no change in the brain serotonin level (Table 7). Therefore the effect of oxotremorine on the brain serotonin level as observed in the present investigation, supports the findings of Cho (1966) and Everett (1964) and is at variance with
the report of Friedman et al. (1963), Wittaker and Walaszek (1964) and Jenden (1968). This difference in observation may be due either to the procedure of assay (biological method employed in the present study) or the effect of other metabolite of tremorine, viz. mono-N-oxide of tremorine, as those workers used tremorine. The increase in level of serotonin in peripheral tissues suggests a role of serotonin for peripheral autonomic effect of oxotremorine. Pretreatment with atropine abolishes the increase in serotonin level of intestine. The efficacy of atropine in antagonising the peripheral effects of oxotremorine (Everett, 1956; Everett, 1963), suggest that the effect on serotonin level in peripheral site and autonomic effect of oxotremorine may be causally related and the rise of serotonin in peripheral tissue by oxotremorine is cholinergically mediated. Role of histamine in the aetiology of Parkinson's disease has been suggested because of the observed effectiveness of antihistaminics in the treatment of true Parkinsonism (Budnitz, 1948; McGavak et al., 1947; Button, 1953). Antihistamines are also found to be beneficial in the treatment of drug-induced Parkinsonism (Smith & Miller, 1961;McGeer et al., 1961; Waugh & Metts, 1960). The brain histamine level is reported to be increased significantly on administration of tremorine (Ungar & Witten, 1963; Wittaker & Walaszek, 1964). This rise of histamine level in brain is mediated...
through cholinergic pathway as atropine abolished this effect (Ungar & Witten, 1963). However Menon et al (1971) have failed to observe any effect of oxotremorine on the histamine level of brain. In the present investigation the effect of oxotremorine on histamine level of peripheral tissues has been studied to evaluate any role of the drug on the histamine level of peripheral sites and any role of histamine in the pharmacological effects exhibited by oxotremorine. The ability of oxotremorine to produce profound changes in the concentration of histamine in blood and tissues as observed in the experiments suggests a role of histamine in drug-induced parkinsonism (Table 8). The fall in histamine concentration after oxotremorine may be due to activation of histaminase (diamine oxidase) resulting in an increased rate of degradation of histamine. However, the discrepancies in the nature of changes in histamine level in blood and intestine/lung rule out this possibility. A significant rise in blood histamine level is observed at 10 min followed by profound fall in the histamine level, which recovers at 160 min.

Release of histamine from the basophils (a circulating counterpart of fixed tissue mast cell) may be the cause of initial rise in histamine concentration of blood. From the results of the present investigation this property of oxotremorine cannot be established. The rapid decline in tissue histamine level after oxotremorine which only partially
recovered after 160 min suggests release of amine from the tissues (intestine and lung) after administration of oxotremorine.

The efficacy of antihistamine in oxotremorine-induced condition in experimental animals and in Parkinson's disease may be due to their local anaesthetic effect (Dutta, 1948) or anticholinergic effect (Reuse 1948). Gerald et al. (1972) have reported that the antioxotremorine effect of various antihistamines are not related to their antihistaminic property but to their anticholinergic property. Therefore an independent role of histamine in the mechanism of parkinsonimimetic effect of oxotremorine cannot be evaluated from the results of the present investigation. But because of the profound changes in the histamine level of peripheral tissues induced by oxotremorine a partial role of histamine in the complex biochemical events involved in the mechanism of action of this drug may be postulated.

The major metabolic effects of oxotremorine, a potent tremorigenic agent and the active metabolite of tremorine, have not been investigated extensively. The results of the present investigation indicate that oxotremorine is a potent glycogenolytic agent and causes both liver and muscle glycogenolysis. The glycogenolytic effects were found to be maximum at 40 min. Gupta and Ganguly (1969) have observed that oxotremorine produces maximal hyperglycaemic effect
at 40 min. Evidently the hyperglycaemia as observed by Gupta and Ganguly (1969) is a result of increased breakdown of liver and muscle glycogen as observed in this investigation. The hyperglycaemic effect of tremorine was also reported by Friedman and Campos (1960) who have suggested this effect of tremorine to be due to centrally mediated release of catecholamines presumably adrenaline, from the adrenal medulla. However, Gupta and Ganguly (1969) have observed hyperglycaemia associated with progressive hypocalcaemia and an increase in inorganic phosphorous level of blood after oxotremorine. The occurrence of hypocalcaemia and increase in inorganic phosphorous level in blood do not simulate the effects of adrenaline (Ellis, 1956, Shim et al., 1968). Thus oxotremorine-induced hyperglycaemia is not likely to be mediated through adrenaline as suggested by Friedman and Campos (1960). The results presented here indicate that the glycogenolytic effects of oxotremorine could not be antagonised by pretreatment of animals with propranolol and DBZ (Table 9). It has been shown that both propranolol and DBZ, in combination are effective in antagonising the metabolic effects of adrenaline relative to hyperglycaemia, glycogenolysis, and lactic acidaemia (Harvey et al., 1952; Yamamura & Horita, 1968). These metabolic effects of oxotremorine are not mediated through release of catecholamines and this observation is at variance with the observation of Friedman and
Campos (1960). However the hyperglycaemia and lactic acidaemia after injection of oxotremorine are suggested to be mediated through peripheral cholinergic activation as both atropine and methylatropine inhibit these metabolic effect of oxotremorine (Oelssner, 1970). The contribution of this metabolic effect to the mechanism of tremorogenic action of oxotremorine has not yet been extensively investigated.

The results, presented here, indicate that the peripheral parasympathomimetic effect and neuromuscular blocking effect are mediated through release of acetylcholine. The decrease of acetylcholine level in peripheral tissues (Table 6) are in conformity with findings on peripheral parasympathomimetic effects of oxotremorine. The antagonism of tremor-response to oxotremorine by HC-3 which inhibits the synthesis of acetylcholine at all cholinergically innervated tissue (MacIntosh et al., 1956) suggests the involvement of the cholinergic system in tremorogenic property oxotremorine (Fig 37). Slater and Rogers (1968) have also reported the antagonism of oxotremorine induced tremor by HC-3 in rats. Bowman and Osuide (1968) have observed the antagonism of tremorine-induced tremor by HC-3 in young chicks, is reported to be unable to pass the blood-brain barrier (Domer & Schuler, 1956).
Slater and Rogers (1968) chose the intraventricular route for administration of HC-3 and Bowman and Osuide (1968) studied the interaction of HC-3 on tremorine - tremor in young chicks with the blood - brain barrier undeveloped. They concluded that the antagonism of tremorine - tremor by HC-3 is mediated through its antagonistic effect on the increase in level of brain acetylcholine by tremorine/oxotremorine. The results described in the foregoing pages (see Results) show that HC-3 extends an antagonistic effect on the peripheral muscarinic effect of oxotremorine as a result apparently of its effect on acetylcholine synthesis since choline, which prevents the synthesis of acetylcholine from being impaired by HC-3, reverses the antagonistic effect of HC-3 (Fig 27a, 33). Moreover, the antagonism of oxotremorine induced tremor by HC-3 in adult mice in which the blood brain barrier is fully developed suggests a peripheral site of action of HC-3 in respect of its antagonism of oxotremorine induced tremor in mice as observed in this investigation. Thus the antagonism by HC-3 of peripheral cholinomimetic effect, neuromuscular blockade at skeletal muscle and oxotremorine-induced tremor in adult mice in which blood-brain barrier is fully developed fairly suggest that besides the central cholinergic system the peripheral-cholinergic system is also possibly involved in the mechanism of tremorogenic action of oxotremorine. The
observations of Leszkovszky and Tardos (1971) on the antagonism of oxotremorine-induced tremor by TK174, a peripherally acting drug which does not pass the blood-brain barrier also support this hypothesis. The antagonism of tremor effect of oxotremorine by morphine (Fig 37) further confirms the involvement of cholinergic system as morphine has been reported to block the release of acetylcholine in brain (Cox & Wienstock, 1966), smooth muscle (Paton, 1957) and skeletal myoneural junction (Pinsky & Frederickson, 1971). The results of the present investigation show that the muscarinic effect at smooth muscle and neuromuscular blockade at skeletal muscle by oxotremorine are mediated through increased release of acetylcholine and these effects of oxotremorine are antagonised by morphine (Fig 34a; Table 4). However, the exact loci of action of morphine as regards its anti-tremor effect cannot be definitely ascertained from the results of the experiments presented here.

The neuromuscular blockade produced by oxotremorine at skeletal myoneural junction is depolarising in nature. The antagonism of neuromuscular blockade produced by oxotremorine by morphine and HC-3 which block the release and synthesis of acetylcholine respectively supports the conclusion that myoneural effects of oxotremorine are mediated through increased release of transmitter substance. d-Tubocurarine when applied microiontophoretically at end
plates of rat, frog and cat muscles in vitro, prevents or reduces the depolarising effect of acetylcholine (Castillo and Katz, 1957a, 1957b, 1957c, 1957d; Thesleff, 1958). The competitive and depolarising type of neuromuscular blocking drugs possess mutual antagonistic effect (Paton & Zaimis, 1949; 1952). Therefore, the antagonism of oxotremorine-induced tremor by d-tubocurarine (Fig 37), a competitive neuromuscular blocking drug, may be due to its antagonistic action on the myoneural effect of oxotremorine which produces a depolarising type of effect at skeletal myoneural junction through increased release of acetylcholine. The dose of d-tubocurarine (50 \mu g/kg) has been chosen so that animals are not incapacitated. Thus the question of antitremor effect by d-tubocurarine because of complete incapacitating paralytic effect may be ruled out. Therefore the antagonism of oxotremorine induced tremor by d-tubocurarine indicates the involvement of skeletal myoneural effect of oxotremorine in the production of tremor.

The antagonism of the oxotremorine induced tremor by drugs e.g. reserpine, amphetamine and imiparine which have in common an interaction with adrenergic nervous system (Agarwall & Bose, 1967; Hammer & Sjoqvist, 1967; Morpugo, 1967) is suggestive of involvement of the adrenergic link in tremorine and oxotremorine tremor. Centrally mediated sympathetic overactivation by oxotremorine at the peripheral site has been reported by Gyorgy et al. (1971). Response of
adrenergic tissue to exogenous adrenaline is potentiated in presence of oxotremorine (Doda et al., 1972). Excitation of central noradrenergic and dopaminergic neurones by oxotremorine have been observed by Corrodi et al. (1967). The results of the experiments on pressor response and isolated hypogastric nerve preparation (Fig. 1, 7 & 12) indicate that oxotremorine induces sympathetic over activation at central as well as peripheral sites. Thus the drugs which have been found to antagonise the effects of oxotremorine involving adrenergic system have been employed to study their effect on the oxotremorine tremor to evaluate the involvement of adrenergic link in the tremorigenic property of oxotremorine. DBZ was found to reduce the intensity of the tremor symptoms induced by oxotremorine. This inhibition of oxotremorine tremor may be either due to the blockade of α-adrenoreceptor or the depletion of tissue noradrenaline, an effect which is brought about by DBZ (Farrant et al., 1964). The antagonism of the oxotremorine induced tremor by bretylium (Boura & Green, 1959) and guanethidine (Fig 38) (Hertting et al., 1962) which reduce the release of noradrenaline from neuronal sites and have been found to reduce the pressor response to oxotremorine in atropinised rats (Fig 3, Table 2) indicate the role of the sympathetic system in the tremor effect of oxotremorine. Reserpine which depletes noradrenaline, dopamine, 5-hydroxytryptamine and gamma-aminobutyric acid (Carlsson, 1965) was
found to abolish tremor response to oxotremorine in mice to a great extent. Oxotremorine-induced sympathetic activation, as observed in hypertensive response and potentiation of responses of vas deferens to nerve stimulation and transmural stimulation, was found to be absent in chronically reserpinised animals. The antagonism of the tremor response to oxotremorine by drugs which were found to antagonise the pharmacological effects of oxotremorine mediated through sympathetic system suggests the involvement of adrenergic system in the tremorigenic property of the drug. Cox and Potkonjak (1970) have also reported the antagonism of tremorigenic effect of oxotremorine by DBZ, reserpine and α-methyl dopa. The increase of serotonin level in peripheral tissue and inhibition of oxotremorine - tremor by cyproheptadine, a serotonin antagonist, suggests some role for this amine in tremorigenic effect of oxotremorine. Oxotremorine is reported to be devoid of any effect on central serotonin neurone (Corrodi et al., 1967) and the results of the present investigation show no change in brain serotonin level after administration of oxotremorine (Table 7). (+) p-Chlorophenylalanine, a specific depletor of serotonin (Koe & Wiessmann, 1966) was without significant effect on the tremor produced by oxotremorine (Cox & Potkonjak, 1970). Therefore the explanation for antagonism of tremor by cyproheptadine may be antagonism of peripheral serotonin receptors. A significant
increase of the serotonin level in intestine was observed after administration of oxotremorine (Table 7). Serotonin is reported to activate the parasympathetic ganglia at smooth muscle (Brownlee & Johnson, 1963) and thereby causes release of acetylcholine. Dretchen et al. (1972) have also reported that serotonin causes release of acetylcholine at myoneural junction. The results presented here, show that oxotremorine produces peripheral muscarinic effects and skeletal neuromuscular paralytic effect through increased release of acetylcholine. Therefore the indirect parasympathomimetic effect of oxotremorine may be partly attributed to rise in the level of serotonin which may cause releases of acetylcholine and antagonism of tremor by cyproheptadine may be attributed to the abolition of this effect of serotonin at peripheral sites. The rapid decline in the histamine level in peripheral tissue on administration of oxotremorine and the reduction of the intensity of tremor response of mepyramine, are not sufficient evidence to be indicative of involvement of histamine in the mechanism of tremorogenic action of oxotremorine, because antioxotremorine effect of various antihistaminics are not attributable to their anti-histaminic property but to their anti-cholinergic property (Gerald et al., 1972). Therefore, the specific role of histamine in tremorogenic effect of oxotremorine cannot be
ascertained from the results of the present investigation. The antagonism of tremor by tolbutamide (Fig 39), a hypoglycemic agent suggest that the metabolic effect of oxotremorine may be partially attributable to mechanism of action of tremorigenic effect of oxotremorine. To sum up the pharmacological effects exhibited by oxotremorine, are manifested by overactivation sympathetic and parasympathetic activity and depolarising type of skeletal neuromuscular blockade. An indirect mechanism is indicated in sympathomimetic, parasympathomimetic and skeletal myoneural effects of oxotremorine. The rapid development of tachyphylaxis of the tremor response to oxotremorine on repeated administration (Fig 36) is also suggestive of an indirect mechanism in tremorigenic effect of the drug.

The antagonism of the tremorigenic effect of oxotremorine by drugs which are known to reduce or abolish the sympathomimetic, parasympathomimetic and skeletal myoneural blocking effect of the drug suggests an adrenergic as well as cholinergic link in tremor response to oxotremorine and some contribution of the effects of oxotremorine at skeletal myoneural junction to its tremorigenic property.