CHAPTER - I.

REVIEW OF LITERATURE
ETIOPATHOLOGY OF PARKINSONISM

Oxotremorine is widely known to produce some of the typical features of Parkinson's disease in experimental animals (Everett, 1956; Farquharson & Johnston, 1959). Moreover it is an extremely potent Parkinsonimimetic agent and this property of the drug is utilised very commonly for screening of therapeutically useful anti-Parkinson drugs (Everett, 1964). It is therefore necessary to discuss the etiopathology and clinical features of the human disease to understand the similarities between experimentally induced condition and the human disease. However, it is beyond the scope of the present review to cover all aspects of human Parkinsonism in this context. Attempts will therefore be made to mention briefly the most relevant features of the human disease.

The extrapyramidal system forms a major part of human brain and plays a most important role in nervous mechanism controlling muscular activity. The extrapyramidal system consists of the following parts: the caudate nucleus, the putamen, the globus pallidus, the subthalamic body of Luys, the red nucleus and the substantia nigra (Best & Taylor, 1961). The caudate nucleus and putamen are often referred to as striatum and the globus pallidus as pallidum.

The basal ganglia however do not have a strict anatomical configuration and the large grey masses at the base of the brain including the optic thalamus have been included by the older anatomists. More recently the thalamus has been excluded and the term is mostly limited to caudate
The chief functions of the extrapyramidal system are described below as narrated by Best & Taylor (1961):

(a) **Voluntary Muscular activity**: Absence of complete paralysis of the contralateral extremities and incapacitating hemiplegia after destruction of corticospinal fibers in the cerebral pedundes (Walker, 1949; Walker, 1952) indicate that voluntary movements in absence of corticospinal fibers are mediated through the co-ordinating function of the multisynaptic system of the precentral motor cortex, basal ganglia and related sub-cortical centres. This is being taken as working hypothesis, the final decisive proof of which is still awaited.

(b) **Reflex muscular activity**: Rigidity is a very frequent manifestation of disease of the basal ganglia, particularly noticeable in Parkinson's disease. Pollock and Davis (1930) have shown that rigidity of paralysis agitans like the spasticity due to decerebration can be abolished if the afferent impulse from the involved muscle to the spinal cord is interrupted. It appears that the extrapyramidal systems have an inhibitory influence on the spinal reflexes which control the muscular activity (Best & Taylor, 1961).
(c) **Automatic associated movements**: Commonly this term is regarded as indicating only the swinging of the arms in walking. This automatic associated movement is abolished by various destructive lesions in the precentral motor cortex, cerebellum, as well as in the substantia nigra and globus pallidus in Parkinsonism (Best & Taylor, 1961).

(d) **Abnormal involuntary movements**: Lesions of different parts of the basal ganglia have been reported to produce various abnormal involuntary movements, the nature of which depends upon the site of lesion. Tremor at resting state as observed in Parkinsonism may be produced by affecting the substantia nigra and globus pallidus (Best & Taylor, 1961).

**MANIFESTATIONS OF EXTRAPYRAMIDAL DISORDER**

Wilson (1912) has made some convincing observations on the relationship of the lesions of basal ganglia to rigidity, abnormal posture and tremor. In the light of these observations the affection of globus pallidus in paralysis agitans described by Jelgersma (1908) and the atrophy of the caudate nucleus in Huntington's chorea (Alzheimer, 1911) gained their actual significance.

(a) **Hepatolenticular degeneration (Wilson's disease)**: Wilson in 1912 described this disease. Its duration varies from a few months to several years, the chief characteristics being muscular rigidity, involuntary movements and cirrhosis of the liver. Involuntary laughing or crying and some mental deterioration are observed.
(b) **Huntington's chorea**: Huntington's chorea is a rare familial disease. The chief characteristic features of this disease are facial grimacing, jerky gesticulating movements of the upper extremities and uncertain gait.

(c) **Double athetosis**: Athetosis is a form of abnormal involuntary movements beginning most often in childhood. The usual clinical symptoms are a bilateral type of athetosis, affecting all parts of the body and particularly the hands, the mouth and the neck.

(d) **Dystonia musculorum deformans**: The striking features of this disease are manifested by dystonia, spasm of varied duration of flexion of the limb and/or torticollis.

An elucidative description of this disorder has been given by Denny-Brown (1960).

**Parkinsonism**: Amongst the extrapyramidal disorders Parkinsonism is by far the most commonly occurring condition which presents many confusing problems.

Symptomatically the disease has been classified under the following headings.

I. **Paralysis agitans or idiopathic Parkinsonism**.

II. **Symptomatic Parkinsonism**.

(a) **Post-encephalitis lethargica**.

(b) **Arteriosclerotic muscular rigidity**.

(c) **Hepato-lenticular degeneration**.
(d) Poisoning by carbon monoxide, mercury, manganese or over dosage with rauwolfia, chlorpromazine and other tranquillising drugs. (Warner, 1964).

The histopathology of Parkinsonism has been excellently reviewed by Denny-Brown (1960). The histopathological changes in the brain in Parkinsonism has been identified as early as beginning of the nineteenth century. These include the reports of Jelgersma (1908) and Lewy (1913) who observed the marked pallor of the ansa lenticularis and various efferent pathways of the pallidum with progressive loss of cells, more marked in pallidum than in striatum. These observations have been further confirmed by many workers (Lhermitte & Cornil, 1921; Bielschowsky, 1922; Denny-Brown, 1960). Considerable loss of cells has also been noted in the substantia nigra in paralysis agitans, and marked damage of substantia nigra in postencephalitic Parkinsonism (Tretiakoff, 1919).

These degenerative changes were found to be distinct in the substantia nigra, globus pallidus, caudate nucleus, putamen and local coeruleus of the pontine tegmentum (Denny-Brown, 1960).

The observations were mostly confirmatory in nature and included considerable pallor of the globus pallidus and the ansa lenticularis in section stained for myelin sheaths.
in all of the cases. The observations also indicated that the loss of nerve fibres was more marked than the loss of nerve cells. The significant findings obtained by Denny-Brown (1960) in his study were characterised by patches of loss of cells and degenerative changes of myelin sheath around outer pallidum near the external lamina. By utilising various histological section techniques, he demonstrated the uneven loss of cells in the pallidum in contrast to the usual evenly scattered pattern of the pallidal cells in normal tissues. The degenerative changes are most marked in the substantia nigra and globus pallidus, widespread atrophic changes also occur, particularly in the cells of the vegetative nuclei of the hypothalamus, brainstem and medulla (Warner, 1964). The melanin containing nerve cells in the brainstem generally show hyalin inclusions (Warner, 1964). Eosinophilic intracytoplasmic inclusions known as 'Lewy bodies', is a consistent feature in Parkinsonism (Den Hartog Jager & Bethlem, 1960; Lipkin et al., 1960; Bethlem & Den Hartog Jager, 1960). This is not limited to basal ganglia but is also observed in the spinal cord and occasionally in the sympathetic ganglia. In very advanced cases of Parkinsonism, histological studies reveal paleness of the pallidal cells which are generally shrunken, and may be filled with lipid in addition to the general outfall of many cells of the substantia nigra and globus pallidus (Denny-Brown, 1963). The skeletal muscle is generally
believed to be unaffected. The lesions of the globus pallidus is described by Bielschowsky (1922) and in detail by Foix and Niculesco (1923) and Denny-Brown (1960) who called it 'paravascular degeneration'. The arteriosclerotic Parkinsonism can be differentiated from what is usually known as true paralysis agitans by associated atherosclerosis of the arterioles and lacunes of the softening around the internal part of the putamen and outer globus pallidus. According to Denny-Brown (1960) the paravascular degeneration is not the result of atherosclerosis but may be worsened by its presence. A detailed understanding of the involvement of different parts of extrapyramidal system in the production of Parkinsonian syndrome has been observed by surgically ablating or producing electrolytic lesions.

Destruction of the caudate nucleus bilateraly in monkey leads to pronounced changes in behaviour, viz. restless pacing movement up and down the backwall of the cage followed by sharplying of the cage but there was no changes in reflexes (Denny-Brown, 1960; Mettler, 1942).

Denny-Brown (1960) successfully produced electrolytic lesions in putamen in two monkeys and the observation suggested that the putamen is responsible for contactile reactions. Electrolytic lesions of the globus pallidus resulted in the loss of all placing reactions. Ranson (cited by Cannon et al, 1944), and Carrea and Mettler (1955) reported that bilateral lesions in the substantia nigra in the monkey has not been associated with the rigidity or
tremor. Denny-Brown (1960) from his experiments on production of selective lesions on the different parts of the extrapyramidal systems in animals concluded that caudate nucleus is responsible for controlling one group of cortical reactions when the other is active. Lesions of the putamen led to athetosis, that is impairment of movement and speech. He also presented evidence of similarity between the lesions of Parkinsonism and the damage of the fiber system of globus pallidus. The activity of the thalamic reflex circuit in the production of tremor and rigidity was shown by Cooper and Bravo (1958) by alleviating these symptoms by inducing lesions of the thalamus. Subsequently it has also been shown that the production of surgical lesions in globus pallidus, ansa and fasciculus, lenticularis, ventrolateral nucleus of the thalamus, substantia nigra or midbrain tegmentum may be helpful in relieving rigidity and resting tremor (Friedman & Everett 1964). Cooper (1961) suggested that ventrolateral nucleus of the thalamus should be the site of choice for surgical therapy of Parkinsonism and this region receives impulses from the globus pallidus, red nucleus, vestibular nucleus and cerebellum and this area also exerts some control on the activity of pyramidal tract and cerebral cortex.

The observations described in the foregoing paragraphs lead to the conclusion that affection of the basal ganglia cause involuntary movements bringing about a disintegration of motor function.
Besides affection of the central nervous system in Parkinsonism, there are also changes in the peripheral regulating mechanism which significantly attribute to the pathophysiology of the disease.

It is established that reflexes regulating the movements and positions of the peripheral muscles through central nervous system is brought about through informations furnished by the receptors present in the skeletal muscles to central nervous system (Best & Taylor, 1961).

The sensory endings in different situations are of the following types: (1) muscle spindles, (2) golgi corpuscles, (3) pacinian corpuscles and (4) unencapsulated nerve endings.

The muscle spindle which is a fusiform body lies parallel to and between the muscle fibers. The muscle spindle is supplied by both afferent and efferent nerves. The efferent fibers are usually small and generally described as gamma (γ) fibers whereas large fibers which supply the rest of the muscle are designated alpha (α) fibers.

The Golgi corpuscles are present in tendons. Afferent nerve fibers enter this organ around its centre and spread upon its adjacent fibers. Any change of tension serves as an effective stimulus to this organ (Best & Taylor, 1961).

The pacinian corpuscles are oval shaped and situated in tendons, joints, periosteum, especially beneath the tendinous insertions, in fasciae covering muscles and in subcutaneous tissue and also in mesentery. These corpuscles
are supplied by afferent fibers which go straight to the centre of the corpuscle. Pressure is the effective stimulus.

Free nerve endings which transmit deep pain are situated between the muscle fibers, in tendons and in fasciae and joints (Best & Taylor, 1961).

Under the physiological condition, the fusimotor system and proprioception are principally responsible for the peripheral regulation of motor activity. Normally muscle spindle is responsible for regulating the length of the mass of main muscle, through the mechanism of 'follow up length servo'. Other feedback mechanisms like Renshaw collaterals which control the frequency of anterior horn cell activity (Struoppler et al., 1960) and the golgi corpuscles exerting inhibitory influences on the spinal cord also modulate the functions of the fusimotor system in a complex way (Hufschmidt, 1960). Friedman and Everett (1964) have stated that the function of the fusimotor system is changed in the production of tremor and rigidity of Parkinsonism. Stern and Ward (1962) and Jung and Hassler (1960) observed that the diminished activity of gamma-loop is responsible for Parkinsonism. On the other hand, the hyperactive 'follow up length servo' has been made responsible for these symptoms (Rushworth, 1960; Barraquer-Bordas, 1958; Pollock & Davis, 1930). Hoffmann (1962) has postulated that the intermittent alpha-firing followed by heavy gamma-efferent driving is the mechanism responsible for
the production of tremor in Parkinsonism. Stern and Ward (1962) have put forward a hypothesis on the basis of their observations and from other related studies that the loss of balance in the alpha-gamma activity resulting from stimulated alpha activity and inhibited gamma activity is responsible for production of tremor, rigidity and akinesia in Parkinsonism.

Therefore Parkinsonism presents a complex picture as regards to its symptoms and etiology. The possibility of the development of a single therapeutically potential anti-Parkinson agent largely depends on the elucidation of the primary cause of the disease. The elucidation of the mechanism of effect of the Parkinsonimimetic agents might contribute to the understanding of Parkinsonism and might furnish in determining an approach to rational pharmacotherapy of Parkinson's Disease.
PARKINSONIMIMETIC AGENTS

Parkinsonimimetic agents are substances which upon administration into animals produce an experimental syndrome resembling somewhat the clinical condition of Parkinson's disease in human beings. A large number of chemical substances, however, are capable of producing tremor, the most outstanding feature of Parkinson's disease. These include alcohol, ether, carbon disulfide, hydrogen sulfide, iodine, bromine, chloral hydrate, carbon monoxide, arsenic, mercury, lead, chromium, zinc, tin, cadmium, copper, thallium, manganese, nicotine, caffeine, opium, morphine, strychnine, curare, quinine, atropine, hyocyamine, colchicine, aconitine, cicutin, veratrin, pilocarpine, camphor, ergot alkaloids, hashish, and poison mushrooms (Friedman & Everett, 1964). But there is no unified theory which can explain the tremorogenic action of these chemically dissimilar substances. Since these chemical agents can not produce the typical picture of the disease, they are not considered to be suitable as pharmacological tools for evaluation of anti-Parkinson drugs. Strictly speaking there is perhaps no chemical agent which can be judged as the ideal. However, some of these agents produce typical Parkinson-like effects in experimental animals and have been found to be of high predictability as models for experimental evaluation of anti-Parkinson drugs. There are considerable differences within these Parkinsonimimetic agents regarding either specificity
or predictability which are important for their ultimate extrapolation for use as experimental models.

Nicotine, harmine, physostigmine, arecoline and tremorine/oxotremorine are generally recognised as useful for this purpose. We shall now, therefore, discuss briefly the pharmacological aspects of these agents individually.

Nicotine: Nicotine has been reported to be of use as a pharmacological tool for evaluating anti-Parkinson drugs (Vernier, 1964; Stumpf, 1962; Bovet & Longo, 1951; Cahen & Lynes, 1951; Everett, 1964). The hyperkinesia and tremor observed after intravenous administration of nicotine, as described by Bovet and Longo (1951) and Cahen and Lynes (1951), have often been used as the experimental technique for this purpose (Jenden, 1968). Intracerebral administration of nicotine has been reported to cause clonic convulsion but not tremor (Molnar et al., 1967). However it is difficult to dissociate the tremor and convulsion induced by nicotine (Vernier, 1964). Studies on the mechanism of tremorogenic action induced by nicotine indicated involvement of peripheral sites in the production of tremor. This was evidenced by the fact that nicotine tremor remained unaltered after section of motor nerve (Everett, 1964). Nicotine fails to produce tremor after intracerebral administration thereby indicating a peripheral site of action. Moreover, dimethylphenyl piperazine, a peripheral nicotine receptor stimulant, has been shown to cause tremor (Molnar et al., 1967). Skeletal neuromuscular junctions may perhaps be the site of nicotine-tremor
as neuromuscular blocking agents like d-tubocurarine, decamethonium, leucaine, procaine and diacetylcholine exhibit marked inhibition of nicotine tremors (Cahen & Lynes, 1951).

The observation of Bovet and Longo (1951) is in variance with the conclusion that nicotine tremor is entirely peripherally mediated. Pentamethylenebistrimethylammonium iodide and tetraethylammonium bromide which antagonise the peripheral action of nicotine failed to suppress the tremors induced by nicotine indicating a central mechanism for nicotine tremor (Bovet & Longo, 1951).

Various substances with different chemical and pharmacological properties have been shown to inhibit the tremor induced by nicotine. Classical anti-Parkinson drugs like diparcol, parpanit, artane, and trasentine exhibited distinct inhibitory effects against nicotine tremor (Cahen & Lynes, 1951). This was further confirmed by Bovet and Longo (1951) who observed a close parallelism between the antinicotinic effect of some drugs and their therapeutic utility in treatment of Parkinson's disease. The Parkinsonian syndrome induced by reserpine and nicotine could be effectively prevented by classical anti-Parkinson drugs, but not by central depressants like phenobarbitone or ganglion blocking agent like tetraethylammonium bromide (Malone et al, 1965).

Vernier (1964) failed to recommend nicotine induced model for screening of anti-Parkinson drugs. His conclusion was primarily based on the observations that central nervous system (CNS) depressants and ganglion blocking agents could
also protect nicotine induced convulsions in mice (Laurence & Stacey, 1952; Stone et al., 1958).

Langley and Dickinson (1809) provided evidence for central origin of nicotine tremor as section of the motor nerves in anaesthetised mammals were effective in preventing nicotine tremors, an observation which is in variance with that of Everett (1964). The central locus of action was further demonstrated by Amantea (1920) and Galamini (1928) who observed motor stimulation after cortical application of nicotine.

Adrenolytic drugs like dibenamine have also been reported to prevent tremor induced by nicotine, 1, 1-dimethyl 4-phenylpiperazinium iodide and acetylcholine (Cahen et al., 1953). The tremor caused by these agents could be due to release of adrenaline. Adrenaline has been shown to possess tremorogenic effect and has also been shown to potentiate tremor in Parkinson patients (Marshall & Schmieden, 1966).

It is therefore apparent that specificity of nicotine induced model for screening of antiparkinson drugs is still uncertain.

Physostigmine: Physostigmine induced model of experimental Parkinsonism has perhaps been least used for screening purpose. It has been suggested that the relative therapeutic utility of anti-Parkinson drug is dependent on their respective cholinolytic effects (DeMaar, 1956; Ahmed & Marshall, 1962). Physostigmine induced experimental model should
therefore he important as a screening method for this purpose. The tremorigenic action of physostigmine after intravenous injection in dogs was found to be superior to that of nicotine or tremorine by Faucon et al. (1965). These authors further observed that physostigmine induced tremors in dogs could be prevented by pre-treatment with classical anti-Parkinson drugs whose relative activity paralleled that of the clinical therapeutic doses.

Physostigmine has been shown to produce tremor when administered into the caudate nucleus (Lalley et al., 1971). Moreover, infusion of acetylcholine into the globus pallidus has been shown to produce tremor (Stern, 1969). It may be presumed that tremor induced by cholinergic agents like physostigmine, carbamylcholine and acetylcholine is mediated through central cholinergic receptors, since central cholinolytic property is most important for beneficial effects of classical anti-Parkinson drugs in clinical use (Friedman & Everett, 1964).

Sinistrotorsion (turning of the head and body to the left) was subsequently developed as a popular method for evaluation of central anticholinergic activity of drugs (Forssman, 1922). Diamant (1954) observed that injection of cholinesterase inhibitors was capable of producing sinistrotorsion. Dejonge and Funcke (1962) first utilised the sinistrotorsion, in unanaesthetized guinea-pigs, induced by injection of physostigmine (0.3 mg./kg.) into the right common carotid artery in a caudal direction, for evaluation
of anti-Parkinson drugs. These authors demonstrated that physostigmine induced sinistrotorsion could be prevented by prior treatment with classical anti-Parkinson drugs like atropine, scopolamine, artane, diparcol, parpanit, cogentin, disipal, benodine and pentobarbital, but remained unaltered after novatropin, buscopan, meprobamate, diphenylhydantoin, reserpine, chlorpromazine, bulbocapnine, LSD-25, tofranil, dl-amphetamine, caffeine and morphine. Atropine methyl nitrate was found to be far less effective in inhibiting physostigmine-sinistrotorsion than atropine and thereby suggesting specificity of central cholinolytic agents to block the sinistrotorsion (DeJonge & Funcke, 1962). Vernier (1964) as well as Friedman and Everett (1964) recommended physostigmine induced sinistrotorsion technique as a superior screening tool for evaluation of anti-Parkinson drugs, since the technique gives good corroborative evidence of their effectivity. The specificity of the technique has however been questioned as pentobarbital and a high dose of iproniazid are also effective in inhibiting the physostigmine induced sinistrotorsion (Friedman & Everett, 1964).

Central catecholamine level has been shown to influence physostigmine-tremor in rats and is evidenced by increased tremorogenic effect after reserpine and chlorpromazine, whereas a diminished effect after L-dopa pre-treatment (Ambani et al, 1972).
The Harmala Alkaloids: For more than a century the Harmala alkaloids are known for their central effects including tremor and rigidity (Gunn, 1935; Chen & Chen, 1939; Friedman & Everett, 1964). Harmine induced tremor in animals has been used as experimental model for screening of anti-Parkinson drugs by many workers (Vernier, 1964; Friedman & Everett, 1964). However the specificity and predictability of harmine induced experimental model is questionable (Vernier, 1964). The antagonism of harmine induced tremor in mice by various chemical agents has been reported by Zetler (1957). The duration of tremorigenic action of harmine is intermediate between tremorine and nicotine (Zetler, 1957).

Attempts have been made by various workers to analyse the mechanism of tremorigenic action of harmine. A central site for tremorigenic action of harmine has been proposed by many workers (Cox & Potkonjak, 1971; Lamarre & Mercier, 1971; Henderson & Woolley, 1970). Studies on ontogenesis of harmine induced tremor have revealed that harmine and harmaline tremor does not occur until 13 days of age and is therefore dependent on development of neural structures (Henderson & Woolley, 1970). This was further confirmed by Hara (1953) who demonstrated that the extrapyramidal action of harmine is related to the development of the brain, the effect being parallel to the development of cerebral cortex. Tremor induced by harmine has been shown to be inhibited by the destruction of cerebral cortex and the corpus striatum (Hara & Kawamori, 1954).
Reports, in variance with above observations, are also available in the literature indicating a direct action of harmine on the extrapyramidal system (Beer, 1939a, b). Studies on neurohumoral involvements in the mechanism of action of harmine indicated some role of 5-hydroxytryptamine and acetylcholine (Kim et al., 1970). These authors observed a significant increase in the level of striatal acetylcholine and 5-hydroxytryptamine in rats after injection of harmine and concluded that tremor like movements and rigidity in the experimental animals are associated with such changes (Kim et al., 1970).

The acetylcholine content of the brain was however found to be significantly decreased after injection of harmine in rats by Cox and Potkonjak (1971), who concluded absence of any cholinergic mechanism in harmine action. Harmine induced tremor in rats were found to remain unaffected by pre-treatment with the monoamine oxidase (MAO) inhibitors (Coates & Cox, 1972).

Information regarding usefulness of harmine induced model for screening purpose has been provided by analysis of the pharmacological activity of harmine antagonists (Zetler, 1957; Cox & Potkonjak, 1971). Various drugs like reserpine, diethyldithiocarbamic acid and propranolol with diverse pharmacological properties have been shown to inhibit the harmine tremor whereas atropine and phenoxybenzamine were found to be without effect (Cox & Potkonjak, 1971).
Zetlar (1957) screened more than 40 compounds against harmine induced tremor and observed protective action by varied type of drugs including anti-Parkinson drugs. A combination of adrenergic blocking, ataractic and anti-5HT activity exhibited highest protection against harmine induced tremor.

The characterisation of harmine induced tremor is therefore complex. Harmine induced syndrome has been rated as intermediate between tremorine and nicotine as regards to its specificity to anti-Parkinsonian drugs by Vernier (1964).

Arecoline: Arecoline, an alkaloid isolated from Areca catechu has long been known as a cholinomimetic agent exhibiting pharmacological properties similar to acetylcholine (Goodman & Gillman, 1970). Like oxotremorine; its a tertiaryamine and both the drugs possess some common pharmacological properties. The peripheral muscarinic effects of arecoline are readily blocked by cholinolytic agents which are impermeable to central nervous system like quaternary salts of atropine (Holmstedt & Lundgren, 1967). The important central effects of arecoline include marked analgesia, tremor, hypothermia, and convulsion (Goodman & Gillman, 1970; Il'Yuchenok & Vinnitskii, 1964; Erkova et al, 1966; Herz, 1962). Anticholinesterase drugs have been shown to enhance both peripheral and central effects of arecoline as characterised by potentiation of tremor and convulsion (Erkova et al, 1966). The tremorogenic, convulsive and analgesic effect of arecoline are inhibited by central
cholinolytic agents like atropine, benactezyne, VVFB-3100 (Vyatchannikov, 1959; Zejmal & Votava, 1961).

Large doses of arecoline are capable of producing nicotinic effects after injection of atropine (Burgen, 1964; Von Euler & Domeij, 1945; Lendle & Ruppert, 1942; Magazanik et al., 1963). The role of the nicotinic effects of arecoline in causing tremor is however not clear. The tremorigenic action of arecoline can be elicited by intracaudate injection of the drug in unanaesthetised cat thereby suggesting a central site of action (Connor et al., 1966). Chalmers and Yim (1962) demonstrated a tremor of about 10/sec with alternating intensity on the right and the left limbs after injection of arecoline in the chronic spinal rats which was relatively short lasting. Procyclidine, a known anti-Parkinson drug could inhibit the spinal tremors of arecoline (Chalmers & Yim, 1962). These authors concluded that arecoline tremor in chronic spinal rats is centrally mediated subjected to feedback control (Chalmers & Yim, 1962). Activation of central muscarinic receptors, therefore, appears to be involved in tremor induced by arecoline.

Tremorigenic action of arecoline has been causally related with changes in the acetylcholine level of the central nervous system by a number of workers. Holmstedt and Lundgren (1966) observed that rise in brain acetylcholine was a constant phenomenon during the tremor caused by arecoline and it lasted approximately for the duration of tremor. Methylatropine neither influenced tremor nor brain
acetylcholine level appreciably. Atropine when administered before or after arecoline inhibited tremor as well as the change in the level of brain acetylcholine. It was further observed that when arecoline was added to an eserinised brain homogenate, there was a rise in the acetylcholine level as compared to control (Holmstedt, 1968). Further evidence of involvement of acetylcholine in the mechanism of thermogenic action of arecoline was provided by Westermann et al. (1970). These authors failed to observe tremor after arecoline in infant and senile rats and in such animals the brain acetylcholine level also remained unaffected. However, both tremor as well as rise in brain acetylcholine level were found to occur in rats aged between 10-30 days.

Attempts have been made to evaluate anti-Parkinson drugs on the basis of their capacity to antagonise the central effects of arecoline like tremor, analgesia etc. (Jenden, 1968). The tremorogenic action of arecoline is of shorter duration with predominant peripheral cholinergic overactivation (Jenden, 1966) which is a disadvantage in using arecoline induced experimental model for screening purposes. However it is evident from the survey of literature that arecoline is an useful pharmacological tool for evaluation of central cholinolytic substances or anti-Parkinson drugs. Other chemical agents of this nature, excluding oxotremorine, are pilocarpine (Jenden, 1968) and aecclidine which are tertiary amines having muscarinic activity (Mashkovsky, 1963).
In the foregoing pages the important pharmacological properties of parkinsonimimetic agents other than tremorine/oxotremorine have been summarised.

Since innumerable reports are available in the literature on the chemical, pharmacological and metabolic aspects of tremorine and oxotremorine, it is perhaps beyond the scope of the present discussion to mention them individually. Important investigations on tremorine and oxotremorine as reported in the literature, are mentioned below with special emphasis on their mechanisms of actions in relation to Parkinsonimimetic effects.

The discovery of tremorine, the metabolic precursor of oxotremorine was first made by Everett and his coworkers in 1956(a,b). The compound was found to be a potent tremorogenic agent with a capacity to produce Parkinson-like features characterised by tremor, rigidity and akinesia in most of the laboratory animals. The action of tremorine was associated with profound muscarinic activation like salivation, miosis, purgation, lacrimation, transient vasodepression and bradycardia etc. The tremorogenic dose of tremorine was observed to be between 5-20 mg./kg. and was dose dependent in nature with a latent period ranging from 5-10 min. Tremor after injection of tremorine was found to occur in most of the experimental species like mice, guinea-pig, rats, dogs, pigeons and monkeys (Parquharson & Johnston, 1959; Friedman &
Smith, 1962; Trautner & Gershon, 1959; Everett, 1956; Everett et al., 1956a; Frommel, 1958). In cats a rage like syndrome and in frogs a sedative effects have been reported to occur after injection of tremorine. Rabbits have been reported to be insensitive to tremorine as regards to its tremorigenic action (Friedman, 1963). But oxotremorine has been shown to cause tremor after intraventricular administration in rabbits and cats (Everett, 1963).

Chemistry and metabolism of tremorine and oxotremorine

1) Chemistry and synthesis of tremorine: The first synthesis of tremorine was reported by Reppe et al. in 1955 and subsequently by other workers (Maier, 1958; Beil & DiPierro, 1958) employing essentially the same techniques. The compound was obtained by condensing pyrrolidine with 1,4-dichloro-2-butyne at room temperature (Scheme).

2) Chemistry and synthesis of oxotremorine: Oxotremorine \[1-(2-oxopyrrolidino)-4-pyrrolidino-2-butyne\] has been synthesised both chemically as well as biologically by various workers (Cho et al., 1961; Kocsis & Welch, 1960; Welch & Kocsis, 1961; Bebbington & Shakeshaft, 1965; Archibald, 1965). Chemically oxotremorine was synthesised by employing N-propargyl-2-pyrrolidone as the intermediate which in turn was obtained by condensation of propargyl halides with 2-pyrrolidone. Further condensation of N-propargyl-2-pyrrolidone with paraformaldehyde and pyrrolidine afforded oxotremorine (Scheme).
**Biological synthesis:** Work on biosynthesis of oxotremorine was initiated by the report of Kocsis and Welch (1960) who presented evidence of biotransformation of tremorine to an activated product responsible for its pharmacological activity. Subsequently the pharmacology of the activated product was also reported by these authors (Kocsis & Welch 1960). The activated product was obtained by incubating tremorine in Krebs phosphate buffer (pH 7.4) with liver slices of mice, hamster or rat at 37° for 2 hours. Filtrate of the incubation mixture when injected into animals exhibited intense pharmacological activity which was different from non-incubated tremorine. The biological activities of the metabolite were characterised by (a) rapid onset of action (b) not being blocked by metabolic inhibitor like SKF 525A (c) stimulation of the isolated intestine and (d) instantaneous vasodepression and bradycardia after intravenous injection. The conversion of tremorine to the
activated product was also found to occur in vivo. This was evidenced as the biological properties of the activated product were also found in the urine of rats and mice after injection of tremorine (Welch & Kocsis, 1961).

Subsequently Cho et al. (1961) undertook further studies on the metabolism of tremorine and reported an elegant route to biosynthesis of oxotremorine from tremorine. These authors also established the chemical identity of the activated product as 1-(2-oxopyrolidino)-4-pyrolidino-2-butyne and named it oxotremorine.

**Metabolic studies on tremorine/oxotremorine:** The observations that tremorine on intravenous injection produced its pharmacological effects after a latent period of few minutes led to the belief that the drug could possibly undergo some biotransformation. It was noted that direct application of tremorine to the brain or the isolated tissue was devoid of any effect indicating the above possibility (Welch & Kocsis, 1961; Jenden, 1968). Subsequently these authors (Welch & Kocsis, 1961) further reported that pre-treatment with β-diethylaminoethyl-2,2-diphenyl valerate (SKF 525A), an inhibitor of the liver microsomal enzymes, prevented the biological activity of tremorine but such pre-treatment did not affect the pharmacological response of activated tremorine. Studies on tremorine/oxotremorine metabolism in chicks indicated participation of several enzymes in the metabolic process (Klinger et al., 1971). Chemical identification of
the active metabolite of tremorine as oxotremorine was made by Cho, et al., (1963) and these authors clearly demonstrated differences between the pharmacological effects of tremorine and oxotremorine. Cho et al., (1964) also presented evidence for existence of an intermediate metabolite namely tremorine-N-oxide which was isolated in vitro by incubation of tremorine with rabbit liver slices. The conversion of tremorine to oxotremorine was suggested to be through the N-oxide in an analogous way (Cho et al., 1964). However the possibility of the N-oxide being reduced to tremorine and subsequently converted to oxotremorine through an independent route has not so far been experimentally refuted (Cho et al., 1964). Tremorine-N-oxide was found to possess similar pharmacological effects like tremorine in all respects.

Existence of other metabolites of tremorine besides tremorine-N-oxide and oxotremorine have been reported by Hammer et al., (1968, 1969). These include N-(4-pyrrolidino-2-butynyl)-γ-amino-buturic acid (Pybu-Gaba) and 1,4-bis (2-oxopyrrolidino)-2-butyne (symmetric dioxotremorine). The isolation and identification of Pybu-Gaba was made from the urine of rats after administration of tremorine. It was also demonstrated that Pybu-Gaba could undergo conversion to oxotremorine in vivo. However, it is not yet established whether Pybu-Gaba is an obligatory intermediate in the formation of oxotremorine from tremorine or whether direct dehydrogenation of hydroxytremorine (Hammer et al., 1968). The
pharmacological actions of Pybu-Gaba closely resembles oxotremorine and has been suggested to be mediated through oxotremorine. 1,4-bis-(2-oxopyrrolidino)-2-butyne (symmetric dioxotremorine), the other metabolite was found to be inactive (Hammer et al., 1969).

Reports on metabolism of oxotremorine are not many in the literature. The instantaneous pharmacological effects like tremor, hypothermia etc. brought about by oxotremorine after intravenous injections, suggests that the drug acts per se. The duration of action of oxotremorine varies widely in different species and is dependent on the age of the experimental animal (Klinger et al., 1971; Zimmermann et al., 1970). Rapid inactivation of oxotremorine has been evidenced by observations on the plasma half of oxotremorine which showed a first order elimination in the perfusion experiments over a wide concentration range. Recently Klinger et al., (1971) provided evidence for enzymatic inactivation of oxotremorine. They demonstrated that liver microsomal enzyme inhibitor like SKF 525A. and thiazinium could inhibit inactivation of oxotremorine. Further it was also shown that pre-treatment with SKF 525A. and thiazinium increased the tremor evoking effectiveness of oxotremorine. There is evidence in the literature that oxotremorine is excreted unchanged in urine after its injection in rats and mice (Jenden, 1968).
1. CENTRAL NERVOUS SYSTEM

The pharmacological activity of oxotremorine has been observed to be most profound on the central nervous system. The central effects of oxotremorine are characterised by profound tremor, rigidity, akinesia, hypothermia, analgesia, ataxia etc. (Jenden, 1968; Friedman & Everett, 1964). The central effects are accompanied by marked parasympathetic stimulation as evidenced by salivation, lacrimation, miosis, diarrhea, bradycardia and instantaneous vasodepression of short duration (Friedman & Everett, 1964).

Of greatest importance out of the central effects of oxotremorine is perhaps the disruption of locomotor activity characterised by sustained generalised tremor, muscular rigidity and weakness, and poverty of spontaneous movement. The tremorigenic effect of oxotremorine is most pronounced in rodents, and in mice the tremor may be so severe so as to disrupt the co-ordinated locomotor activity (Jenden, 1968). The frequency of tremor induced by oxotremorine varies widely depending on the species, being 20-24 c.p.s. in mice and 10-15 c.p.s. in rats. In larger species, the central effects are frequently masked by profound peripheral muscarinic activation leading to cardiovascular collapse. However the central effects may be unmasked in such cases by premedication with quaternary anticholinergic substances (Jenden, 1968).
tremorogenic action of tremorine and oxotremorine are less marked in cats and rabbits (Jenden, 1968). It has been reported that oxotremorine produces a rage like syndrome in cats characterised by fear and aggression (Koff & Langfitt, 1966) and rarely a coarse, resting tremor. On the other hand Nash and Emerson (1959) has reported tremor after tremorine in cats which occurs at a frequency of 8-12/sec.

Peripheral sympathetic overactivation characterised by contraction of the nictitating membrane after administration of oxotremorine has been reported by Gyorgy et al., (1971). This property of oxotremorine is mediated through a central mechanism.

George et al.,(1962) have studied the central action of oxotremorine in a variety of species viz. chickens, pigeons, mice, rats, guinea-pigs, rabbits, cats, dogs and monkeys. All the animals were pre-treated with methylatropine to eliminate the peripheral effects of oxotremorine. Besides, ataxia, tremor and spasticity, all the animals exhibited some type of central stimulatory effect. There were symptoms like pacing, jumping and circling followed by rage-like syndrome in cats and monkeys. The rage was followed by fear or withdrawal reaction. There was some excitatory effect of cerebral cortex evidenced by hallucinatory experiences.

These results suggested a central stimulatory effect of oxotremorine. It has also been reported to possess a central analgesic effect which is 500 times more than morphine (Jenden, 1968). George et al.,(1966) suggested that the
tremorigenic effect of oxotremorine was due to its central sites of action by experiments with differential transection of central nervous system. Transection through caudal midbrain and anterior pons prevented tremor on systemic administration of oxotremorine (George et al., 1966). Electrolytic lesion in rostral to midbrain and caudal pons abolished oxotremorine tremor but lesions of diencephalic structures failed to alter the tremorigenic effect. The central site of action of oxotremorine was also detected by Kaelber and Hamel (1961) who observed that electrolytic lesions in the region of posterolateral hypothalamus and ventromedial thalamic nuclei either significantly raised the threshold or produced blockade of tremorine induced tremor in 10 out of 23 cats. Tremorine, when injected into globus pallidus, caudate nucleus and substantia nigra, produced tremor of higher intensities than control (Cox & Potkonjak, 1969b).

Zetler (1968) observed a cataleptic state after injection of oxotremorine in mice pre-treated with methyldroprine. Since this could be antagonised by atropine, he concluded that this property of oxotremorine was centrally mediated. Intracerebroventricular administration of small doses of oxotremorine in conscious mice has been shown to produce tremor, hypothermia and cholinergic overactivation (Ankier et al., 1971). Involvement of peripheral β-adrenoceptor mechanism has been indicated by Ankier et al. (1971) in the hypothermic property of oxotremorine.
The hypothermic effect of tremorine is believed to be a central effect as it is antagonised by atropine but not by methyl atropine (Spencer, 1965). Lomax and Jenden (1966) have shown direct effect of tremorine on the thermoregulatory centre of preoptic nucleus.

Large number of reports are available in the literature on interactions of other drugs with tremorine and oxotremorine. A brief mention will now be made of such reports which are of particular interest.

Tranquillizers like reserpine, chlorpromazine, asarone and β-asarone have been reported to partially inhibit the tremorine-tremor (Friedman & Everett, 1964; Dandiya & Menon, 1965). The mechanisms of tremor-inhibitory action of these drugs seem to be different. Depletion of biogenic amines has been postulated to be responsible for antitremorine action of reserpine (Jenden, 1968). Anticholinergic effects of asarone, β-asarone & chlorpromazine have been suggested to be responsible for their antitremorine action. (Ryal, 1956; Kopera & Armitage, 1954; Dandiya & Menon, 1965). But the true efficacy of phenothiazines against tremorine-tremor has been questioned in view of their inhibitory effects of liver microsomal enzymes (Leslie & Maxwell, 1964). Central anticonvulsants and local anaesthetics are devoid of antitremor activity (Friedman & Everett, 1964).

Imipramine has been reported to inhibit the hypothermic effect of oxotremorine to a greater extent than tremor
(Spencer, 1965). Spencer (1966) has also reported that amphetamine selectively inhibits hypothermic effect without altering the tremorigenic effect of oxotremorine. Moreover the tertiary but not quarternary atropine derivatives have been reported to inhibit hypothermic response to tremorine (Spencer, 1965). In addition, Ferrari and Gessa (1964) have observed preferential blocking effects of anti-Parkinson drugs like orphenadrine, ethopropanazine and diethazine on tremor and parasympathetic effects but not on the hypothermic effect of tremorine. These authors have concluded that possibly tremorine acts on two systems, one responsible for tremor which is sensitive to atropine, scopolamine as well as anti-Parkinson drugs, and the other responsible for thermoregulation sensitive to atropine and scopolamine (Ferrari & Gessa, 1964).

Employing classical anti-Parkinson drugs, Ahmed and Marshall (1962) have demonstrated a close parallelism between antitremorine and antiacetylcholine effects for these agents.

Analgesia is an important feature of central action of tremorine and oxotremorine (Jenden, 1968; Everett, 1964). The analgesic effect of oxotremorine is manifested at comparatively lower dose level than the tremorigenic effect (Haslett, 1963; Chen, 1958). Physostigmine has been reported to potentiate the analgesic effect of a relatively higher dose of oxotremorine (Handley & Spencer, 1969). Anti-Parkinson
drugs which inhibit tremor also antagonise the analgesic effect induced by oxotremorine.

Propranolol, a β-adrenoceptor blocking agent has been shown to inhibit the tremor induced by tremorine and oxotremorine (Leslie et al., 1972; Agarwal & Bose, 1967; Cox & Potkonjak, 1970). The mechanism of antitremor action of propranolol is however disputed. Sharma (1970) has attributed this action of propranolol to be related through its β-adrenolytic property. At the same time it has also been suggested by other workers (Marsden et al., 1967) that action of propranolol is mediated through unknown peripheral propranolol-sensitive receptors. Beta-adrenoceptor blocking activity of propranolol seems to be unrelated to its antitremor action (Agarwal & Bose, 1967). The importance of skeletal neuromuscular apparatus in relation to anti-tremor action of propranolol is discussed later.

It is interesting to mention that phenoxybenzamine, an α-adrenolytic agent, inhibits the tremor and parasympathomimetic effects of oxotremorine (Cox & Potkonjak, 1970). The mechanism of antitremor action of phenoxybenzamine is uncertain (Farrant et al., 1964).

To sum up, the reports on effects of oxotremorine and their interaction with various drugs clearly indicate the involvement of autonomic nervous system in the mechanism of action of tremorine/oxotremorine. There is evidence of simultaneous participation of both adrenergic and cholinergic
systems in the mechanisms of tremorigenic actions of these drugs. It is also apparent that peripheral mechanisms play an important role in bringing out the Parkinson-like effects of tremorine and oxotremorine.

**Spinal cord:** The involvement of central nervous system in the mechanism of action of tremorine is supported by the observation that there is loss of tremor below the level of spinal transection in animals (Everett et al., 1956). This observation was at variance with that of Nash and Emerson (1959) who observed tremor and spasticity at all levels (above and below the section) in cats maintained for 2 weeks after complete spinal section. This again could not be substantiated by Kaelber and Hamel (1960) who failed to observe any response below the transection using the same technique. Subsequently Chalmers and Yim (1962) have shown that tremorine is able to induce tremor in chronic spinal rat when the animals are recovered from spinal shock. On the basis of this observation these authors suggested involvement of the spinal cord in tremorine - tremor.

Jurna et al. (1970) have examined the $\alpha$ and $\gamma$ reflex activity in relation to induction of tremor and rigidity by tremorine and oxotremorine. Both tremorine and oxotremorine increased $\alpha$-reflex activity leading to rigidity which is manifested by appearance of toxicity of the muscle and potentiated the $\gamma$-activity characterised by rhythmic $\gamma$-discharge.
It has also been observed that drugs which antagonise tremor also abolish the potentiated $\alpha$ and $\gamma$-activity induced by tremorine and oxotremorine. Thus there may be a casual relationship between tremorigenic effect and stimulatory effects of tremorine and oxotremorine or $\alpha$ and $\gamma$-activity (Jurna et al., 1970). On the other hand Decima and Haslett (1964) have observed that injection of tremorine and oxotremorine into mesencephalic reticular formation blocks the gamma fiber activity. Blazevic et al. (1967) have also demonstrated participation of $\alpha$-cells of the anterior horn in the mechanism of action of tremorine. Activation of $\alpha$ and $\gamma$-motoneurone after injection of tremorine has been demonstrated by Forcier and Tasker (1969). These observations provided evidence of participation of spinal reflex mechanism contrary to the general concept of supraspinal activation by tremorine (Forcier & Tasker, 1969).

Baker (1963) has studied the effects of tremorine on motor reflexes in lightly anaesthetised cat. Tremorine at a dose of 10 mg/kg produced facilitation of patellar reflex and also was found to antagonise the inhibition of patellar reflex induced by ipsilateral stimulation of sciatic nerve. It also caused depression of lingnomandibular reflex. All these effects of tremorine could be antagonised by atropine (Baker, 1963). However tremorine has not been found to affect the polysynaptic flexor reflexes. On the basis of his observations Baker (1963) has concluded
that the facilitation of patellar reflex by tremorine is mediated centrally via enhanced spinal motor neuronal discharges. Chalmers and Yim (1963) have reported that tremorine (4 mg/kg) potentiated the flexor and crossed extensor reflexes. The elevated reflex responses and tremor are antagonised by anticholinergics and anti-Parkinson drugs.

Extrapyramidal: Ablation of the caudate nucleus has been found to raise the threshold of tremorine-tremor (Bernhang et al., 1958). This observation implicated the involvement of caudate nucleus in the production of tremorine - tremor. In variance with the observation of Bernhang et al. (1958), it has been reported by Blazevic et al. (1965) that surgical ablation of caudate nucleus fails to modify the tremorine - tremor. But the unilateral ablation of globus pallidus causes the reduction of intensity of tremor when tremorine is injected in rats. Local injection of oxotremorine in globus pallidus has been shown to produce instant tremor whereas injection into caudate nucleus is without effect (Blazevic et al., 1965).

2. AUTONOMIC NERVOUS SYSTEM

Tremorine produces profound cholinergic activity in addition to its Parkinsonimimetic effects like tremor, rigidity, asthenia etc (Everett, 1956). The autonomic effects of tremorine and oxotremorine are characterized by salivation, bradycardia, hypotension, blockade of effects of vagus nerve stimulation on heart, increase in contraction
and tone of the urinary bladder, facilitation of transmission of the superior cervical ganglion, a marked transient mya-
driasis in mice, negative inotropic and chronotropic action on the isolated rabbit atria, inhibition of erythrocyte and intestinal mucosal cholinesterase, stimulation and blockade of transmission through inferior mesentric ganglion in cat (Friedman & Smith, 1962). Oxotremorine is reported to be as potent as acetylcholine in muscarinic activity both in vivo and in vitro (Cho, Haslett & Jenden, 1962).

The peripheral muscarinic effects of oxotremorine are independent from its central effects as methyl atropine which cannot pass the blood brain barrier selectively antagonises the muscarinic effects of the drug (Levy & Michel-Ber, 1965).

In short, oxotremorine mimics the peripheral muscarinic activity of acetylcholine. However the nicotinic effects of acetylcholine like rise of blood pressure (b.p.) in atropinised rats and contracture of striated muscles have been reported to be absent after oxotremorine injection (Levy & Michel-Ber, 1967; Cho, Haslett & Jenden, 1962). It is interesting to mention that a stimulant action on the superior cervical ganglia has been reported by De-Groat and Volle (1963) following close arterial injection which is blocked by atropine. The mechanism of this action of oxotremorine has been suggested to be due to a direct excitatory action on atropine sensitive sites in sympathetic ganglia. The mechanism of cholinergic
overactivation by oxotremorine is still not definitely known. The available reports suggest involvement of both direct and indirect mechanisms. Increase in the level of brain acetylcholine, as reported by various workers (Holmstedt & Lundgren, 1966; Pepou, 1963; Holmstedt et al., 1963), has indicated a possibility of indirect action mediated through its effect on acetylcholine metabolism. The rise in brain acetylcholine after tremorine and oxotremorine has been reported to last approximately for the duration of tremor. Moreover atropine, but not methyl atropine, injected either before or after tremorine and oxotremorine, has been found to abolish tremor as well as to prevent the increase in brain acetylcholine. The effects of oxotremorine on cholinacetylase are not uniform. Stern and Gasparovic (1962) have reported activation of cholinacetylase after tremorine, an observation not in agreement with that of Holmstedt et al. (1965). Similarly it has also not been possible to demonstrate any specific effects of tremorine and oxotremorine on acetylcoenzyme A, a vital enzyme for the synthesis of acetylcholine (Schuberth et al., 1965). It is therefore difficult to attribute increased biosynthesis of acetylcholine as the underlying mechanism for rise in brain acetylcholine after tremorine and oxotremorine. Moreover there is no evidence of cholinesterase inhibition after tremorine and oxotremorine except at very high concentrations (Cho et al., 1962; George et al., 1962; Holmsted et al., 1965).
There are increasing evidences in the literature suggesting enhancement of acetylcholine release by oxotremorine. The concept of increased release of acetylcholine to bring about the peripheral cholinergic action of oxotremorine stands against the generally believed direct postsynaptic effect of the drug at cholinergic receptors (Cho et al., 1962; Haslett, 1963; Levy & Michel-Ber, 1967). The arguments in favour of an indirect mechanism for central effects of both tremorine and oxotremorine have been put forward by Bowman and Osuide (1968) and by Slater and Rogers (1968). These authors have successfully demonstrated that tremor response to tremorine is depressed by compounds like hemicholinium-3 and triethylcholine which inhibit acetylcholine synthesis.

Recently Gyorgy et al. (1970) have demonstrated that the peripheral effects of oxotremorine on smooth muscles may be inhibited by incubation with hemicholinium-3. Evidence for indirect mechanism of action of oxotremorine at other peripheral sites like neuromuscular junction is also available in the literature (vide Neuromuscular Effects). Attempts have been made by several authors to correlate the antitremor (induced by tremorine and oxotremorine) and antiacetylcholine effects of various classical anti-Parkinson drugs. But the results are not very uniform. Farquharson and Johnston (1959) have failed to observe any correlation between antitremor and antiacetylcholine activity, whereas Ahmed and Marshall (1962)
have observed a distinct relationship between the two by employing various anti-Parkinson drugs.

Tremorine and oxotremorine have also been shown to affect the ganglionic site. Friedman and Smith (1962) have demonstrated that ganglion blocking agents inhibit the parasympathetic effects of tremorine and have suggested that autonomic effects of tremorine are largely due to its effect on peripheral autonomic ganglia.

Levy and Michel-Ber (1963) have observed antinicotinic effect of tremorine without parasympathetic properties on isolated rat duodenum and guineapig auricular myocardium. Moreover effect of tremorine on eserinised rat duodenum has been found to be similar to that of nicotine (ganglio-excitatory and ganglioplegic).

β-adrenergic blockers particularly propranolol have been found to antagonise the pharmacological effects of tremorine and oxotremorine (Leslie et al., 1972). Propranolol has also been reported by other workers to cause inhibition of oxotremorine induced tremor (Agarwal & Bose, 1967; Cox & Potkonjak, 1970; Jacobi, 1957). But the central effects of oxotremorine like analgesia and hypothermia are not affected by propranolol (Hermansen, 1968).

Recently from this laboratory it has been shown that antioxotremorine action of propranolol is perhaps mediated through its effect at skeletal neuromuscular junction (Ganguly, 1973).
Transient mydriasis, a generally believed peripheral effect of oxotremorine (Friedman & Smith, 1962) has been shown to be inhibited by centrally active drugs like reserpine and tetrabenazine. Since local application of oxotremorine fails to produce this effect (Phan et al., 1972) it has been suggested that oxotremorine induced mydriasis is perhaps centrally mediated.

In addition, imipramine and amitriptyline, the two well known psychoanaleptic agents, have been shown to abolish the cholinergic effects of oxotremorine (Achari & Sinha, 1968; Levy & Michel-Ber, 1965).

It is therefore apparent from the survey of literature that reports on the mechanisms of autonomic effects of oxotremorine are diverse and dissimilar. In the present investigation, attempts have therefore been made to re-examine the underlying mechanisms of peripheral autonomic effects of the drug.

3. NEUROHUMORAL

Profound neurohumoral changes have been observed by many workers after injections of tremorine and oxotremorine. In such studies, special emphasis has been given to unearth a correlation between tremorigenic action and alterations of biogenic amines induced by tremorine and oxotremorine. These studies are of particular significance in view of derangement in the metabolism of biogenic amines in Parkinson's disease (Barbeau, et al., 1961; Hornykiewicz, 1966; Barbeau, 1966).
Significant changes in the level of biogenic amines in the brain have been reported after injection of tremorine characterized by decrease in the level of norepinephrine (rat, mouse, guineapig) and a significant increase in the level of 5-hydroxytryptamine (Friedman, 1963, Friedman, et al., 1963). Tremorine has been reported not to alter the catecholamine level of the rabbit brain and it is interesting to mention that tremorigenic effect of tremorine is meagre or absent in this species (Friedman et al., 1963). Depletion of norepinephrine by oxotremorine from adrenergic and dopaminergic nerve terminals has been observed after previous inhibition of tyrosine hydroxylase by Corrodi et al. (1967). Further, an activation of adrenergic and dopaminergic neurones under the influence of oxotremorine has been observed which is perhaps mediated through a cholinergic link (Corrodi et al., 1967). Norepinephrine level in the brain also diminishes after tremorine injection in chick (Bowman & Osuide, 1967). But no noticeable change in the norepinephrine level of the whole brain in rat has been observed by Whittaker and Walaszek (1964) except when the neocortex is excluded, in which case a small but significant decrease in the norepinephrine is observed after injection of tremorine. In variance with this observation, there are several reports available in the literature indicating that tremorine and oxotremorine do not affect the levels of biogenic amines in the brain. Anton et al. (1967) have failed to observe any alteration in the levels of brain norepinephrine and dopamine.
after administration of tremorine but there was significant depletion of cardiac norepinephrine. Treatment of rats with tremorine and oxotremorine has been shown not to produce any change in the levels of norepinephrine, epinephrine, dopamine and dopa by Everett (1964) and Holmstedt and Lundgren (1966).

Similar is the dispute as regards to effects of tremorine and oxotremorine on the 5-hydroxytryptamine level in the brain. Whittaker and Walaszek (1964) and Jenden (1968) have demonstrated increase in the level of brain 5-hydroxytryptamine after tremorine in rats. Attempts have even been made to correlate the rise in 5-hydroxytryptamine level after tremorine with its tremorogenic action (Jenden, 1968). But no change in the brain 5-hydroxytryptamine level has been observed after injection of tremorine and oxotremorine by other workers (Everett, 1964; Cho, 1966).

It is therefore difficult to draw any conclusion from these diverse and dissimilar observations on the mechanism of action of tremorine and oxotremorine.

Reports are also available in the literature regarding changes in brain histamine levels after tremorine and oxotremorine. Ungar and Witten (1963) have reported that histamine level of whole brain of rats, and striatum and hypothalamus of dogs are significantly increased after injections of tremorine. This has been further confirmed by Whittaker and Walaszek (1964) who have demonstrated that
the histamine level of whole brain is elevated by about 40% in rats when treated with tremorine. Increase in the histamine level after tremorine has again been suggested to be through a cholinergic pathway, as atropine blocks this effect (Ungar & Witten, 1963). Recently Menon et al. (1971) have failed to observe any alteration of brain histamine level even after toxic doses of tremorine. It is necessary to mention in this connection that pre-treatment with 4-thiazolyl-methaoxamine (TMA), a potent depletor of brain histamine does not produce any change in either intensity or duration of tremorine induced tremor. Moreover the protective effect of various antihistamines as reported by Gerald et al. (1972) are perhaps due to their anticholinergic properties unrelated to their antihistaminic activities (Gerald et al., 1972). Thus it is difficult to implicate a specific role of brain histamine in tremor induced by tremorine and oxotremorine.

Significant and valuable observations are provided by Holmsted et al. (1963) and Pepeu (1963) as regards to causal relationship between alterations of brain acetylcholine level and tremor produced by tremorine and oxotremorine. Rise in acetylcholine content of brain appears to be a constant phenomenon after injection of tremorine and oxotremorine which coincides with the duration of tremor induced by these agents (Holmstedt & Lundgren, 1966). The change in brain acetylcholine level after tremorine and oxotremorine has been reported to be inhibited by atropine
According to Bartolini et al. (1970), the rise in the level of cholinergic neurotransmitter is most pronounced in the extrapyramidal areas of the brain like caudate nucleus, substantia nigra and globus pallidus. Cox and Potkonjak (1969b) have observed tremor after direct injection of tremorine in these areas. The exact mechanism by which oxotremorine and tremorine increases the brain acetylcholine level is not definitely known. The possibility of stimulated synthesis of acetylcholine by tremorine and oxotremorine is remote as has been discussed earlier. The hypothesis put forward by Holmstedt and Lundgren (1966) that oxotremorine may mobilise acetylcholine from some unidentified storage site needs further confirmation.

Cox and Potkonjak (1969a) have not been able to correlate the change in brain acetylcholine and tremor in a critical analysis. It may be concluded from the overall survey of the literature that tremorine and oxotremorine may bring about Parkinson-like effects through an indirect mechanism on both central and peripheral cholinergic system. Release of acetylcholine from central and peripheral storage site after tremorine and oxotremorine appears to play an important role in the mechanism of action of these drugs.

4. NEUROMUSCULAR

Reports on involvement of skeletal myoneural junction in the mechanism of action of tremorine and oxotremorine are not
many. It has been generally believed that tremorine and oxotremorine are devoid of any effect at neuromuscular junction (Cho, Haslett & Jenden, 1962; Levy & Michel-Ber, 1967).

The important and interesting observation of Csillik (1964) on the myoneural effect of tremorine has been remaining obscure for a considerable period. It has been shown that tremorine treatment in rats induces a kind of calcium release in the motor end plates similar to that induced by acetylcholine-like agents. Since the same effect results even if the appropriate motor nerve has previously been transected, it is concluded that this myoneural action of tremorine is not due to a central mechanism but to a peripheral stimulatory property of the drug (Csillik, 1964). Subsequently Levy and Michel-Ber (1967) have been able to detect a curare-like paralytic effect of oxotremorine on the rat phrenic nerve-diaphragm preparation. A depolarising and subsequent desensitising effect of oxotremorine has been demonstrated by Elmqvist and McIssac (1967) in the chronically denervated rat hemidiaphragm preparation in vitro. The results of Elmqvist and McIssac (1967) have indicated a direct depolarisation of the muscle end plate in presence of oxotremorine. More recently Ganguly and Choudhuri (1970) have provided evidence for an indirect action of oxotremorine, but not of tremorine, on the skeletal myoneural system which are partially in accordance with the observation of Csillik (1964). Lower concentration of oxotremorine has been shown
to produce spontaneous fasciculations and a higher concentration of oxotremorine inhibits the neuromuscular transmission (Ganguly & Chaudhuri, 1970). These authors have also reported a profound effect of oxotremorine on the cat sciatic nerve anterior tibialis muscles in vivo. On the basis of these observations, an indirect mechanism of oxotremorine at myoneural site has been suggested (Ganguly & Chaudhuri, 1970).

5. METABOLIC

A few reports are also available in the literature on the metabolic effects of oxotremorine. The significance of these observations in relation to the tremorigenic property of the drug is however not very clear. Oxotremorine has been reported to produce hyperglycemia in rats originally by Friedman and Camphos (1960). The hyperglycemic effect of oxotremorine has been initially suggested to be due to centrally mediated release of catecholamine from the adrenal medulla. Subsequently Gupta and Ganguly (1969) have confirmed the hypoglycemic effect of oxotremorine, together with increase in inorganic phosphorus as well as progressive fall in the level of calcium in rat blood. These metabolic effects of oxotremorine are not similar to those observed after adrenaline and are therefore in variance with the observations of Friedman and Camphos (1960). Recently Oelssner et al. (1970) have suggested that the hyperglycemia and lactic acidemia after injection of
oxotremorine are mediated through cholinergic mechanisms. This suggestion has been made in view of the fact that previous treatment with both atropine and methyl atropine could inhibit these effects of oxotremorine. Since methyl atropine is able to prevent the aforesaid effects, a peripheral cholinergic activation appears to be more likely.

Significance of observations like diminished total flavine content and decrease in iron content of brain after tremorine and oxotremorine (Stern & Hasanagic, 1967) are still obscure.

For summary the pharmacological effects of oxotremorine are diverse and views regarding its mechanism of action are also divergent and dissimilar. These include (a) direct activation of central muscarinic receptor and other central mechanisms (Cox & Potkonjak 1969a; Everett, 1964; Kaelber & Hämäläinen, 1960), (b) indirect cholinomimetic mechanism — (Holmstedt & Lundgren 1966 & Pepeu 1963) and (c) participation of central and peripheral sympathetic nervous system (Bose & Agarwal, 1967; Achari & Sinha, 1967; Cox & Potkonjak, 1970). Moreover the observations of myoneural involvement of oxotremorine (Csillik, 1964; Ganguly & Chaudhury, 1970; Elmqvist & McIssac — 1967; Ganguly, 1973; Leszko & Tardos, 1971) are of importance as the skeletal muscle is the target organ for production of tremor and rigidity.
In the present investigation, special attempts have been made to elucidate the involvement of cholinergic, sympathetic and biogenic amines in the mechanism of Parkinson-like effects of oxotremorine. Elucidation of the mechanism of oxotremorine is expected to provide a more specific model for evaluation of newer and novel anti-Parkinson drugs as oxotremorine is widely used as a pharmacological tool for this purpose.