SYNOPSIS

INTRODUCTION:

Progress in drug therapy is often measured by the development of agents which are selective and specific in their effects to replace older. They must have negligible untoward effects. The lack of truey specific neuromuscular blocking agent has been felt most keenly in the production of muscular relaxation during surgical anaesthesia. Apart from this, their use in anaesthesia these drugs are also useful in the control of convulsive seizures of tetanus, muscular spasticity, in the prevention of trauma in electroconvulsive therapy and in orthopaedic maneuvers.

d-Tubocurarine, a widely used neuromuscular blocking drug which causes histamine release and ganglion blockade producing precipitous fall in blood pressure, Gallamine produces tachycardia. The production of deep muscle pain as an after effect restricts the use of Succinylcholine in the clinical practice. Irreversibility of Decamethonium and the absence of
antagonist out bars its use. Dantrolene sodium, recently introduced drug specific for skeletal muscle spasticity produces drowsiness, dizziness, weakness, general malaise, fatigue and diarrhoea.

'Tubocurarine' the first neuromuscular blocking drug was obtained from the plant Chondrodendron tomatosum while 'Erythroidine' with curariform property was obtained from the plant Erythrina americana. Synthetic processes, for which a chemist employs heat and pressure, are affected in plants at ordinary temperature and pressure, e.g. quinine was synthesized by chemist after intensive work extending over half a century whereas the Cinchona plant synthesizes in without difficulty everyday. What is in store Nature alone knows. Research on plants should therefore go on in the interest of humanity. Thus it is worth tapping the natural source further while searching for new neuromuscular blocking drugs.
In the preliminary work carried out on the plant *Iris germanica* (Orris root, Iridaceae) it is found to possess neuromuscular blocking activity. Hence it was thought to probe into its potentiality as a neuromuscular blocking activity, using various tests and also to isolate, identify and elucidate the structure of the active principle showing neuromuscular blocking activity.

Orris root or rhizome as the drug is commonly termed is derived from three species of *Iris, Iris germanica, Iris florentina, Iris pallida*. The rhizomes of these three species closely resemble each other. It is reported\(^3\) that Oris rhizome contains myristic acid together with Irone, an oily liquid, with a powerful odour of violets. The rhizome also contains crystalline glucoside-iridine.

Oris rhizome is described to be a stimulant, emetic, cathartic and diuretic\(^4\). Iridine—a toxic glucoside is isolated from several species\(^5\). An aqueous extract of *I. germanica* decreases smooth muscle activity in vivo. It was devoid of toxicities and psychotrophic effects in mice\(^6\).
The work is divided into two parts:

1. Chemical work comprised of isolation, identification and structural elucidation of biologically active compound.

2. Pharmacological work consisted of the evaluation of neuromuscular blocking activity of the isolated compound using battery of tests, both in vivo and in vitro employing various animal species.

Chemical work:

The dried plant material (rhizome of Iris germanica) was powdered and extracted sequentially with petroleum ether (40-60°C), chloroform and methanol. The preliminary pharmacological work on isolated frog rectus abdominis muscle preparation and isolated rat phrenic nerve diaphragm preparation indicated that the petroleum ether fraction is more potent in exhibiting neuromuscular blocking activity and hence the remaining
plant material was extracted with petroleum ether and this extract was used to isolate the active fraction.

During the process of isolation at every stage, fractions were screened for neuromuscular blocking activity using isolated rat phrenic nerve diaphragm preparation and the fractions showing neuromuscular blocking activity were pursued further.

**Method of isolation and separation:**

Petroleum ether extract was concentrated under reduced pressure. The concentrated extract (oily, about 60 gms) was chromatographed on column using silica gel G as absorbent and benzene and chloroform as eluting solvents. The percentage of chloroform was gradually increased from 0% to 20%. Thin layer chromatography of crude extract on silica gel G as adsorbent and 100% chloroform as solvent system yielded four spots after spraying with 50% Sulfuric acid. TLC was adopted for monitoring the purity of fractions. In the column chromatography four fractions were isolated, Fraction I (13 gms), Fraction II (7 gms-CBIF-54),
Fraction III (10 gms) and Fraction IV (50 gms).
Fraction II i.e. CBIF-54 is an oily substance and was found to be biologically active and hence chemistry of this compound was carried out in detail. It was soluble in hexane benzene, ethylacetate, chloroform, partially soluble in acetone methanol and ethanol. It was insoluble in water. In order to elucidate the structure of CBIF-54, various spectral analysis such as IR, UV, NMR and Mass Spectra were carried out.

Fraction CBIF-54 shows positive test for Dragendorff's reagent.

**Pharmacological work:**

The potential of CBIF-54 as a neuromuscular blocking agent was evaluated employing a battery of *in vitro* and *in vivo* tests in various species as mentioned below:
In vitro preparations:

1. Isolated dorsal muscle of leech
2. Isolated rectus abdominis muscle of frog.
3. Isolated phrenic nerve diaphragm of rat.
4. Isolated biventer cervicis muscle of chick.
5. Isolated lattissimus dorsi muscle of chick.
6. Isolated cremaster muscle of guinea pig.

In vivo preparations:

1. Anterior tibialis nerve muscle preparation of cat, rat and guinea pig.
2. Intravenous administration in conscious animals viz. mouse, rat, chick and rabbit.

CBIF-54 exhibited mixed type of neuromuscular blocking activity in all the in vitro and in vivo preparations. It exhibited reversible blockade of acetylcholine induced contraction on isolated frog rectus muscle and isolated guinea pig cremaster muscle at lower doses (50 μg/ml) and flaccid paralysis after intravenous administration in conscious chicks (lower doses, 0.5 & 1 mg/chick). These observations favour
antidepolarising type of action while depolarising type of action was shown by *per se* contraction of isolated frog rectus, leech dorsal muscle (at higher doses) and chick biventer cervicis muscle, initial fasciculations followed by block by indirect stimulation of isolated rat phrenic nerve diaphragm preparation and spastic paralysis in conscious chicks (dose 2-4 mg/chick, i.v.). The block produced by indirect stimulation of rat phrenic nerve diaphragm preparation was dose dependant and was neither potentiated nor inhibited by physostigmine. The block produced by CBIF-54, by direct muscle stimulation of curarized rat diaphragm and its antagonism by calcium chloride shows resemblance with dantrolene sodium. This block was slow in onset.

To sum up, a pure compound (CBIF-54) was isolated from rhizomes of *Iris germanica*. The structure determination of this compound is in progress. The compound exhibited mixed type of neuromuscular blocking activity. It different from α-tubocurarine in its neuromuscular blocking activity in the fact that physostigmine failed to antagonize the blockade caused by CBIF-54 on isolated rat phrenic nerve diaphragm
preparation. The onset and duration was dose dependant and is also produced Wedensky's inhibition, like that of d-tubocurarine. CBIF-54 caused per se contraction of chick biventer cervicis muscle and it produced reversible spastic paralysis when administered intravenously in conscious chick, lasting for about 5-10 min. with immediate onset of action. This action is like that of decamethonium. CBIF-54 caused block of direct muscle stimulation of curarized rat diaphragm, and was antagonized by increasing the concentration of calcium chloride in bathing fluid. This action of CBIF-54 is like that of dantrolene sodium.

The effect of CBIF-54 on smooth muscle and on cardiac muscle was also studied. It produced dose dependant spasmodic action on guinea pig ileum at higher doses. This was partially blocked by atropine. It caused positive inotropic effect on isolated frog heart.

So in this new compound we have got a structure which is exhibiting actions of the three types of peripheral muscular relaxants viz. antidepolarising, depolarising and directly acting.