PART I

GENERAL CONSIDERATIONS
PHARMACOLOGICAL EVALUATION OF A NEW SERIES
OF SYNTHETIC LOCAL ANAESTHETIC

COMPOUNDS

PART I

General Considerations

Section 'A': Introduction, brief review of the local anaesthetics drugs and aim of the present study:

Periodically during the course of medical history discoveries have been made which influenced greatly the course of medical practice. The discovery of local anaesthetics and its production is of great value. Accidentally in 1860, it was observed that the alkaloid, cocaine, when placed on tongue, causes sensation of numbness. Von Anrep discovered in 1879 that subcutaneous injection of a solution containing cocaine causes loss of sensation at the site of injection; he suggested that this might have practical value in medical practice. A few weeks after, Halstead, in United States begun investigations which resulted in the discovery of nerve block anaesthesia with cocaine. Cocaine was thus the first drug to be used as a local anaesthetic, though cocaine possesses several desirable properties as local anaesthetic, its toxicity and tendency to cause addiction became known soon after, thus limiting its clinical
application.

The introduction of procaine by Einhorn in 1905 as local anaesthetic agent, eliminated the problem of addiction associated with cocaine and reduced the danger of severe intoxication with it by a significant extent. Since Einhorn's discovery of procaine, literally thousands of synthetic agents have been tested, with the result that substances now are available which represent practically all degree of potency, duration of action and for that matter toxicity too.

Xylocaine (Lignocaine) is one such synthetic local anaesthetic which was introduced by Lofgren in 1946. The chemical structure of xylocaine is entirely different from the existing synthetic local anaesthetics. Its properties were studied for the first time by Goldberg (1949) and Wielding (1952). It is about twice as potent as procaine by injection and is fairly effective on intact mucosa on rabbit's cornea, it is about 8 time more potent than procaine, twice as potent as monocaine, but only 2/3 as potent as cocaine. It is also suitable for spinal anaesthesia. Its toxicity for guineapig is 1/4 to 1/3 greater than procaine (Frommel et al. 1950).

Introduction of a new chemical structure in xylocaine opened up an entirely new field for research in the synthetic local anaesthetics. In order to secure more potent and non-toxic local anaesthetic drugs, analogues of xylocaine
were prepared by Trivedi & Dalai (1960). Some of these were selected and investigated for thorough pharmacological investigations and form the subject of this thesis.

When a new drug is discovered, natural or synthetic, it has to be evaluated very carefully and critically regarding its pharmacological action, toxicity and therapeutic potentialities. Therefore in the present study the newly synthesised analogues of xylocaine were subjected to very rigorous test from these points of view. Local anaesthetics as a group when come in direct contact with nervous tissue, cause its paralysis. The susceptibility of the various nerve fibres however, prevents marked and characteristic quantitative differences. Sensory fibres are most easily attacked. By using appropriate dilutions complete paralysis can be achieved, which is however, reversible, if the drug is washed away or absorbed and the nerve fibres recover their functions promptly and completely.

In order to assess the local anaesthetic activity of drugs, number of methods may be employed as have been described by different workers from time to time. Some of these are mentioned below:

1. Intracutaneous injection - the 'wheal' or 'quaddel' method of H. Braun (1914).

2. Surface application of the solution of local anaesthetics on the mucous membranes. The skin of frog's
foot is more convenient, but rabbit's or guineapig's cornea corresponds more closely to clinical conditions (Chance & Lobstein 1944).

3. Plexus anaesthesia in frog. The results correspond to the conditions of 'nerve block' (Munch et al. 1933).

4. Paralysis of the motor fibres of the excised nerve trunk of frogs by local anaesthetic solutions. It gives concordant results, especially when evaluated by nerve action potentials (Bennett et al. 1942).

5. The human intradermal wheal method.


7. Rat-tail conduction block anaesthesia (Herret et al. 1954).

The results obtained by using different methods may often vary. Herret et al. (1954) compared the local anaesthetic activity by using different methods and found that the infiltration and conduction potencies of the various local anaesthetics generally run parallel. Conduction anaesthesia however requires higher concentrations than infiltration because the difficulty in penetrating the myelencephalic sheath.

In addition to their ability to block the transmission of nerve impulses, the local anaesthetics have several other actions in common, such as:-

1. Central stimulation followed by depression and death due to respiratory failure.

2. Action on the myoneural junction and ganglionic
synapse.

3. Action on the cardiovascular system, causing vasodilation and antiarrhythmic action by a few drugs such as procaine.

4. Action on the smooth muscles, producing an anti-spasmodic action etc, etc.

In view of these general properties of synthetic local anaesthetics it was considered worthwhile to investigate the general pharmacological actions of the present series of local anaesthetics. Hence, while studying the local anaesthetic potency of these compounds their actions were investigated thoroughly on cardiovascular system, smooth muscles, skeletal muscles, central nervous system and respiratory system. Acute and chronic toxicity was studied in mice and rats respectively.

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SECTION 'B' - CHEMICAL CONSIDERATION.

The development of local anaesthesia did not hit its stride until the discovery of the local anaesthetic properties of cocaine. This alkaloid can be obtained by extraction of Erythroxylon coca and other species of Erythroxylon. Properties which detract from the medicinal value of cocaine are its high toxicity, its addiction liability and cortical stimulation. These properties together with the relatively high price of cocaine, the danger of its administration and the legal restrictions imposed on the drug on account of its addiction liability have led to an intensive search for non-habit forming, less toxic and possibly more potent and longer lasting local anaesthetic. In spite of the fact that this research has produced literally hundreds of compounds with local anaesthetic activity of which many could be used clinically cocaine is still used in ophthalmology because of its mydriatic action and in dilating swollen nasal passages prior to sinus lavage.

Evolution of synthetic Local Anaesthetics:

(1) In order to prove the significance of the tropane ring system for the local anaesthetic effects of cocaine, several simple derivatives of piperidine containing only six-numbered ring of the condensed tropane system have been tested. Of special interest are \( \alpha \) & \( \beta \) Eucains (Harries 1897, 1903).
(2) Ritsert in 1943 introduced ethyl p-amino benzoate as a local anaesthetic. Einhorn modified this molecule and got orthoform (Methyl -3 hydroxy -4-amino-benzoate) and orthoform new (4-hydroxy-3-amino-benzoate). This was a very important lead in the development of local anaesthetics.

(3) A combined knowledge obtained from the degradation of cocaine and the activity of the alkyl p-amino benzoate enabled Einhorn to proceed to the effective series of local anaesthetic, dialkyl amino alkyl esters of aromatic acids. The cocaine molecule can be visualised as consisting of an aromatic acid, esterified with a tertiary amino alcohol.

\[ \text{H}_2\text{C} \quad \text{CH} \quad \text{CH}_3\text{COOCH}_3 \quad \text{N}\text{CH}_3 \quad \text{CHO} \quad \text{CO} \quad \text{C}_6\text{H}_5 \]

By combination of this group with that present in the p-amino benzoates, a series of compounds were prepared of which procaine had the most favourable therapeutic ratio (Einhorn and Uhlfelder, 1909.)

\[ \text{H}_2\text{N} \quad \text{COOCH}_2 \quad \text{C}_2\text{H}_2 \quad \text{N}\text{C}_2\text{H}_5 \text{N}_2 \]

The drawbacks of procaine are its relatively weak anaesthetic action by pharmacological standards and the
short duration of its action.

(4) The replacement of fundamental oxygen atom by the amino group can lead to isosteric compounds of similar properties. Searching for antipyretics in the acetanilide series the analogous cyclic compound, oxindole and its ring homologue dihydro-carbostyril were tested. This study led to the discovery of an important local anaesthetic Dibucaine (Miescher, 1932).

Among the amide type of anaesthetics Lidocaine has become one of the most satisfactory local anaesthetics because of very few side - reactions (Erdtman & Lofgeren, 1937).

Several para substituted analogoues of Lidocaine were prepared. The p-ethoxy derivative was more active and less toxic than procaine.
This indicates that ortho substituents are not absolutely necessary for the development of anaesthetic activity (Hofsterter et al. 1953).

Benzyl group seems to play an important part in the molecule of many drugs having activity on nervous system. Kushner et al have shown that Hibicon - (N-benzyl- B-chloro propionamide -(Cl.CH₂.CH₂.CO.NH.CH₂ C₆H₅) exhibits a strong anticonvulsant activity. Dimethoxy benzyl group is present in the well known antispasmodic papavarine present in opium.

Goldman & Williams in 1953 found that benzyl esters of certain 4-carbamyl-1-piperizine carboxylates were active anticonvulsants.

Hibital, N-benzyl Nicotinamide and Lispamin contain in their molecule benzyl group and they show powerful antispasmodic activity (Suter et al. 1948). Dibenzyl acetic acid ester of diethyl amino alcohol is found to be most effective antispasmodic -

\[ \text{C}_6\text{H}_5 \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{C}_6\text{H}_5 \cdot \text{COOCH}_2 \cdot \text{CH}_2 \cdot \text{NH} \cdot \text{C}_2\text{H}_5 \]

(Wegner et al. 1939).

It was thought that it would be interesting to prepare basic-amides in which NH₂ group is separated from the aromatic ring by one or more carbon atoms. Such type of reasoning has led in past to the discovery of important drugs.
Hence it was decided by Trivedi et al (1962) to test basic amides containing benzyl group. With this object in view dialkyl amino, - morpholinyl - and piperidinyl, - N-benzyl acetamides and - propionamides were prepared by the methods described below.

**General method of preparation**

**Hydrochloride of secondary amino N**(substit-benzyl)** acetamides.**

1(a) Preparation of N-Subst Chloro acetamides

To the benzyl-amine (1 g) in 5 ml. cold water, cooled in ice-salt mixture, was added drop by drop with vigorous shaking, chloro acetyl chloride, and simultaneously 10% NaOH (5 ml) solution. After allowing the
reaction mixture to attain room temp., the precipitate was filtered, washed with a little cold water, mixed with HCl (1:1) and finally with water. The chloroamides thus obtained were then crystallised from dil. alcoholic.

(b) Preparation of secondary amine derivative of amide.

The secondary amine is prepared by refluxing for five hours. The above prepared acetamide (1 mol.) in 500 ml. dry benzene with (2.6 mol) secondary amine (diethyl amine or morpholine or piperidine). The precipitated secondary amine hydrochloride is filtered off and washed with benzene. The residue was dissolved in a little more than the calculated quantity of 3 N HCl (~340 ml.).

After filtering, the solution was purified by shaking with ether and then made alkaline with ammonia. The liberated base was then taken up in ether and dried over MgSO₄.

(c) Preparation of Hydrochloride of secondary amino N-(Subst. benzyl) alkyl amides.

In the above prepared ethereal solution of the amide dry HCl gas is passed, when Hydrochloride of the amide is precipitated. It was filtered and in dry acetone form colourless needles.

General method for the preparation of Hydrochloride of secondary amino N(Subst. benzyl) propionamides.
2(a) Preparation of \( \text{w-Subst. halo propionamides.} \)

Prepared by the method shown in 1 a. in which benzyl amine is treated with \(< - \text{ halo - propionylhalide}. \)

Preparation of secondary amine derivative of propionamide.

(b) Prepared as shown in method 1 b. by refluxing secondary amine with \( \text{N-subst. halo propionamide in benzene.} \)
Preparation of Hydrochloride of secondary amine derivative of Propionamide:
Prepared as shown in method 1.c.

17 compounds were screened for local anaesthetic action and the following table lists these compounds in the decreasing order of potency.

2. diethyl amino N-2-Chloro benzyl acetamide.
3. Piperidinyl N-3-Chloro benzyl acetamide.
4. Piperidinyl N-4-bromo benzyl acetamide.
5. Piperidinyl N-4-chloro benzyl acetamide.
7. diethyl amino N-3-chloro benzyl acetamide.
8. Piperidinyl N-4-methyl propionamide.
11. morpholinyl N-4-chloro benzyl-propionamide.
12. morpholinyl N 2:4 dimethyl benzyl acetamide.
13. diethyl amino N-4-methoxy benzyl-propionamide.
15. Morpholinyl N-3-chloro benzyl acetamide.
16. Morpholinyl N-4-bromo benzyl acetamide.
17. diethyl amino N 2:4 dimethyl benzyl-propionamide.

Out of these 1, 2, 3, 10, 11 & 12 were selected for detailed study. The results were compared with Lignocaine, Cocaine and Procaine for local anaesthetic activity. These compounds are designated as under:
Chemical structure of compounds selected for detailed study

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Chemical structure and code</th>
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5. Hydrochloride of Morpholinyl-N-(4-chloro benzyl) propionamide.