INTRODUCTION

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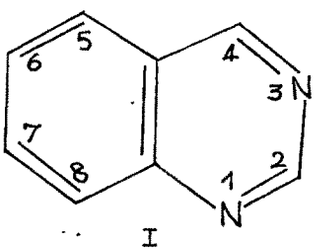
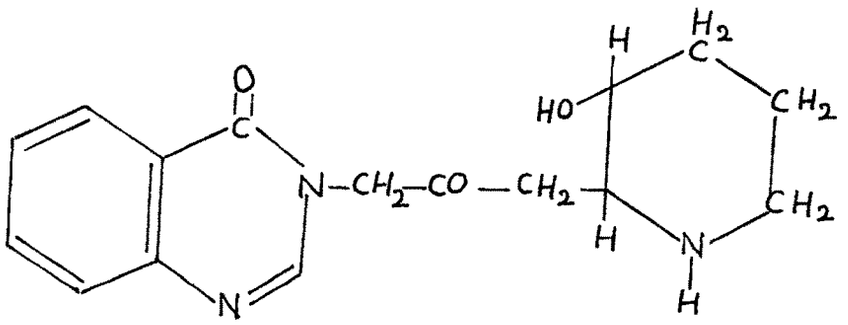
Alkaloids possessing quinazoline (I) nucleus as part of their structure were in therapeutic use since ancient times. A preparation, isolated from the roots of Dichroa febrifuga Lour, was in use in China under the well-known name of 'Ch'ang San', from as early as 200 B.C. (Liu et al, 1941; Jang et al, 1944; Koepfli et al, 1950). Febrifugine (II) and isofebrifugine (III) were found to be the active principles of this anti-malarial preparation (Koepfli et al, 1950).



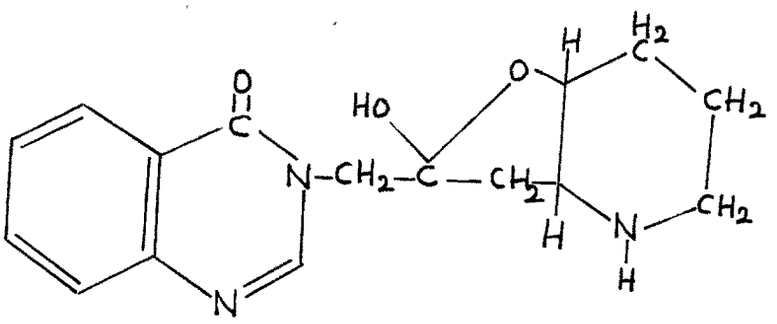
I

Quinazoline



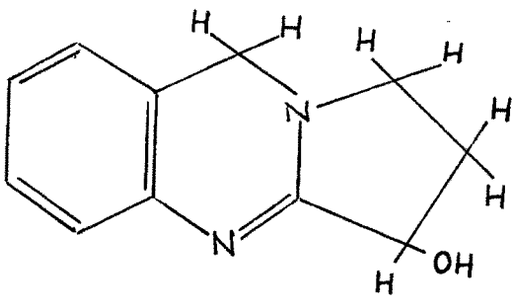
II

Febrifugine



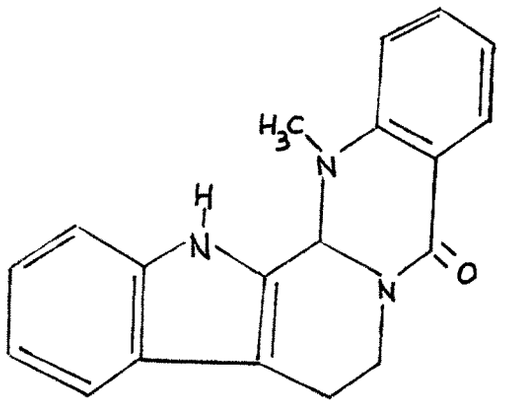
III
Isofebrifugine

Vasicine (IV), another quinazoline alkaloid was first isolated by Hooper in 1888 from the leaves of the Indian plant Adhatoda vasica Nees, locally known as 'Arusa' and was recommended for a variety of ailments such as bronchitis, asthma, fever and jaundice. A preparation made from the leaves and roots of this plant was found to exert an antispasmodic effect also (Manjunath, 1948).

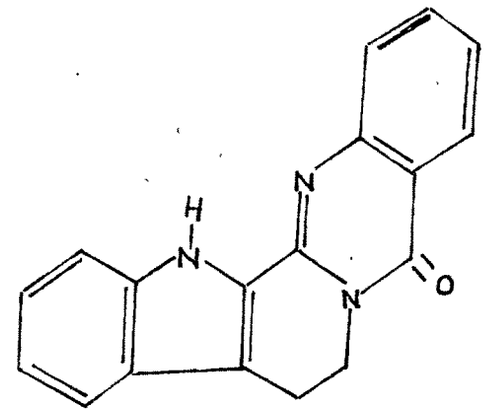


IV
Vasicine

Evodiamine (V) and rutaecarpine (VI) alkaloids containing both quinazoline and indole nuclei, are well known and isolated from Evodia rutaecarpa Benth and Hook (Asahina, 1915; Pachter et al, 1960).



V
Evodiamine



VI
Rutaecarpine

Quinazoline derivatives were found to differ in their biological effects depending on the nature and position of the substituents. Their actions range from antibacterial-antimalarial to central nervous system (CNS) depressant and anticarcinogenic ones. A few of these derivatives, both natural and synthetic and their properties are summarized in Table-I.

Table-I

	Trivial name	Chemical name	Properties	Reference
1.	Vasicine[b]	1,2,3,9-Tetrahydro-pyrrolo(2,1-b) quinazoline-3-ol	Used in bronchitis, asthma, fever and jaundice	Hooper (1888).
2.	Methaqualone[a]	2-Methyl-3-o-tolyl-4(3H)-quinazolinone	Hypnotic, anticonvul-sant	Gujaral et al (1955, 1957)
3.	Quinethazone[a]	7-Chloro-2-ethyl-6-sulfamoyl-(4H) quinazolin-4-one	Oral diuretic, antihyper-tensive	Novello (1961); Cohen and Vaughn (1961).
4.	-[a]	2,4-Diamino-quinazolinone. α-bis(2,4-diaminoquinazolyl-6-amino)p-xylene	Potent dihydro-folate reductase inhibitor	Hutchison (1968); Hynes et al (1972).
5.	-[a]	2-Methyl-3-(4-methoxybenzyl-idinoamino)-4-(3H)-quinazolinone	Tuberculo-static	Muraveva (1971)
6.	-[a]	2,4-Diamino-6-[2-(3,4-dichloro-phenyl)acetamido]-quinazolinone	Inhibitor of RNA and protein synthesis in C. neo-formans-184	Hariri and Larsh (1976)

a - synthetic; b - natural source

Some chemical properties of the quinazolines and their derivatives are given in the following pages. A detailed review is also available (Armarego, 1963).

Peter Greiss (1869) was the first to report the synthesis of quinazoline (I) in the laboratory. Widdege (1887) proposed the name "Quinazolinone" which has also been called as phenoniazine; benzo-1, 3-diazine; 5,6-benzpyrimidine.

4-hydroxyquinazolines have high-melting points and are water-insoluble substances. They are readily soluble in alkali and form stable salts (Körner, 1887; Widdege, 1887; Paal and Busch, 1889 and Söderbaum and Widman,1889). 4-hydroxyquinazolines are most conveniently obtained by the Niementowski reaction (Niementowski, 1895) whereby anthranilic acid is fused with an aliphatic amide. This reaction has been used extensively and the yields are generally over 50% (Baker et al, 1952; Ben and Singh, 1959; Armarego, 1963).

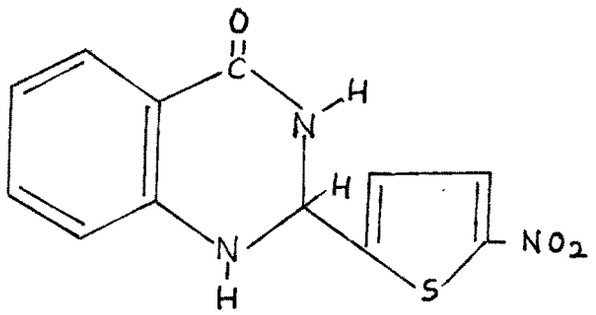
2,3-disubstituted-4(3H)-quinazolinone derivatives can be synthesized from o-acylamidobenzoic acid with the required amine in presence of phosphorous trichloride or oxychloride (Mewada et al, 1955; Salimath et al, 1956). o-Acylamidobenzoic acid esters have also been used in this type of reaction (Dallacker et al, 1960).

The 4-hydroxyquinazolines in general show strong ultraviolet absorption and the spectra of some of the substituted quinazolines in polar solvents or in acids are sufficiently characteristic to be useful for their quantitative or qualitative assay in biological materials (King and Perry, 1969).

Biological Effects:

Of interest to the present investigations are compounds such as 2,3-disubstituted quinazolinones. Compounds in this group such as 2,4-diamino-, hydrazino- and 5-chloro quinazolines have been reported to exert inhibitory effect on the growth of microorganisms (Niepp *et al*, 1957; Asano and Asai, 1958; Baker *et al*, 1958; Hutchings *et al*, 1960; and 1961; Hutchison, 1968). Similarly, sulphanylamino derivatives have also been reported to exert tuberculostatic activity (Muraveva *et al*, 1971).

Alaino and Russel (1972) have studied the action of 2,3-dihydro-2-(5-nitro-2-thienyl)-quinazoline-4(1H)-One (VII) against *Streptococcus aureus*, *Streptococcus faecalis*, *Corynebacterium liquifaciens*, *Escherichia coli* (two strains), *Salmonella typhosa* and *Hemophilus vaginalis*.



VII

2,3-dihydro-2-(5-nitro-2-thienyl)-quinazoline-4(1H)-One

A comparative study of methotrexate and some 2,4-diaminoquinazoline analogs (several of which were found to be more inhibitory than methotrexate) was presented by Hutchison (1968). Their structural similarity with folic acid is shown in Fig.1. Their relative inhibitory effects in Streptococcus faecalis (var.durans SF/0) as well as on several sublines of the L_{1210} mouse leukemia and in cultured L_{1210} cells were reported in detail by him.

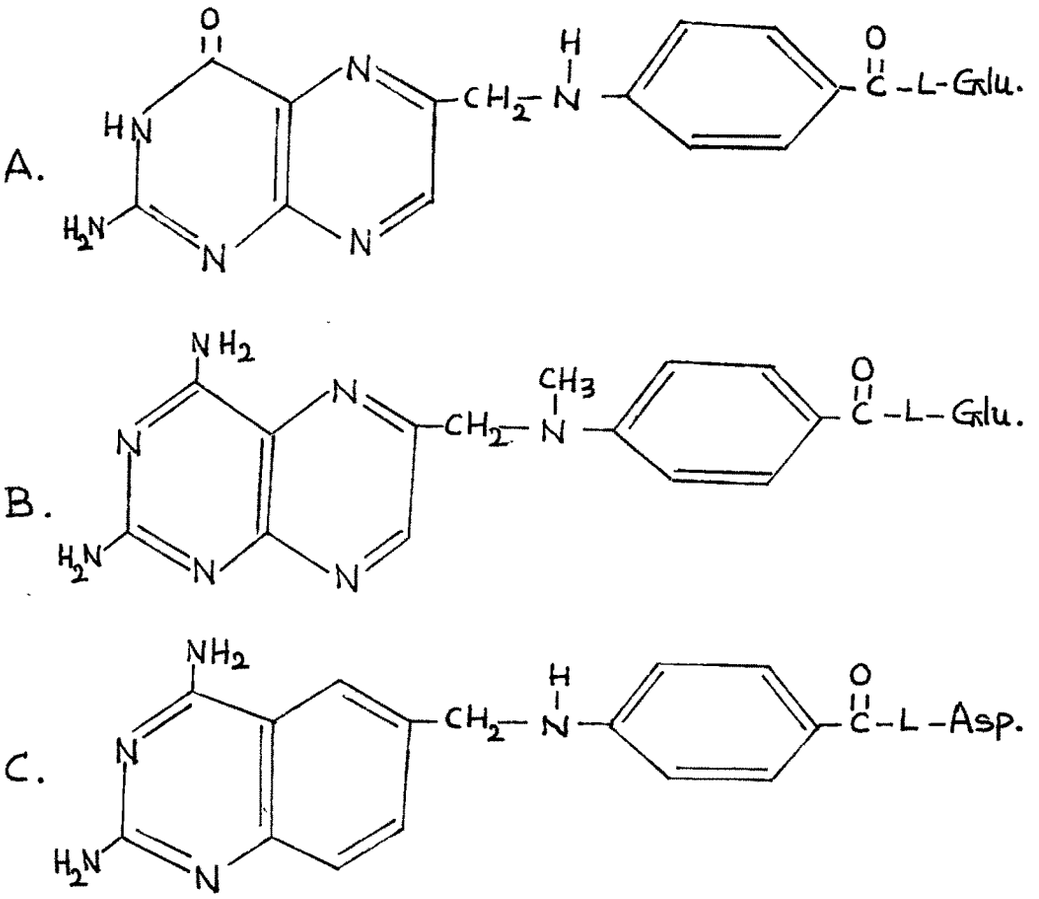
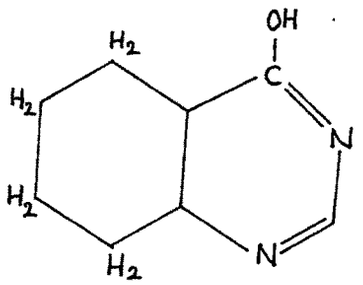


Fig.1: Folic Acid (A), Methotrexate (B) and Quinazoline Analog (C)

L-Glu. = L-Glutamic acid

L-Asp. = L-Aspartic acid

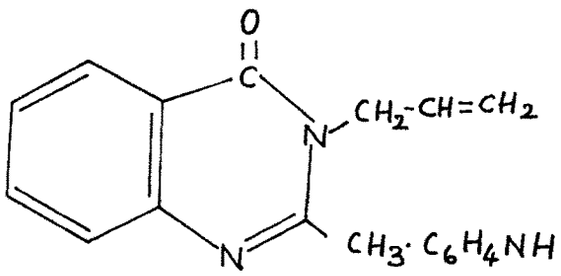
Another derivative, 4-hydroxy-5,6,7,8-tetrahydroquinazoline (VIII) has been reported to possess fungicidal activity (Margot and Gysin, 1958). Activity *in vitro* and *in vivo* of 2,4-diamino-6-[2-(3,4-dichlorophenyl) acetamido]- quinazoline was seen against *Cryptococcus neoformans*-Strain 184, the causative agent of European blastomycosis (Hariri and Larsh, 1976).



VIII

4-hydroxy-5,6,7,8-tetrahydroquinazoline

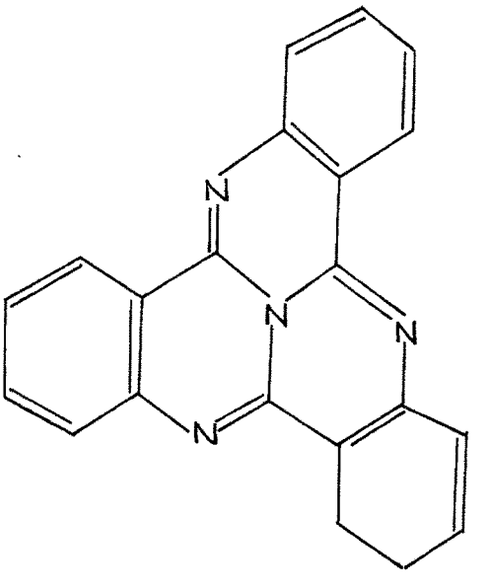
The effect of this antifungal agent on macromolecular metabolism was also reported and it was found that this compound inhibited the incorporation of labelled precursors such as ^{3}H-Uridine or ^{3}H-Leucine into ribonucleic acid (RNA) or into proteins of *C. neoformans* respectively. An alkaryl derivative (IX) was reported "to be 100% effective at 3 parts per million (p.p.m.)" against powdery mildew (Bullock and Sheeran, 1975).



IX

A variety of quinazoline had been surveyed for their antimalarial activity (Wiselogle, 1946; Baker, 1958 and Baker and Querry, 1958). A 3-w-N-morpholylpropyl derivative and a 3-ω-N-piperidyl-n-butyl-quinazoline derivative were shown to have significant antimalarial activity (Magidson and Lu, 1959). Several 2,4-diamino quinazolines were also reported as potential antimalarial agents (Hynes et al, 1972).

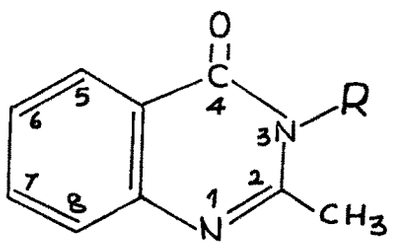
Cooper and Partridge (1954) found that tricyclo-quinazoline (X) is a stronger carcinogenic agent in mice (72%) than in rats (27%). Its action resembled that of embedded plastic materials, because it was recovered unmetabolized in the urine (Baldwin et al, 1959; see also Cooper and Partridge, 1954).



X

Tricycloquinazoline

Structure of the two synthetic derivatives (XI) used in this study are given below.



XI

Methaqualone, R = o-tolyl

SRC-820, R = 3-methylpyridine

Compound SRC-820, a 2-methyl-3-(3'-methyl-2' pyridyl)-4(3H)-quinazoline-4-One was reported to share many of the pharmacological properties with 2-methyl-3-o-tolyl-4(3H) quinazolinone (Methaqualone) such as hypnotic, tranquilizer, anticonvulsant, antipyretic and analgesic action[1]. It appears that introduction of substitutent to

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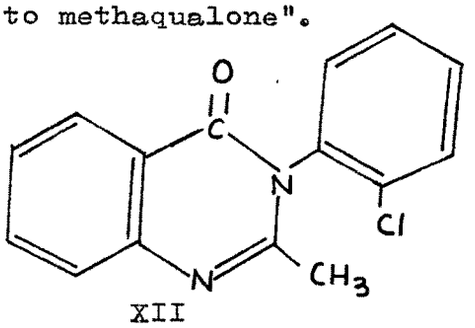
1 Results of Studies reported from Sarabhai Research Centre, Baroda.

the quinazoline nucleus or reduction of the substance to the dihydro derivatives resulting in loss of CNS depressant activity but retains their analgesic properties (Boltze et al, 1963). Replacement of the 2-methyl radical by fluoro or hydroxy or amino groups does not affect the hypnotic properties. On the other hand, the anticonvulsant activity could be enhanced with the loss in hypnotic activity by replacing the 2-methyl substituent with larger, alkyl ones with 4-amino group (Boltze et al, 1963; Bonati and Rosati, 1965; Breuer and Roesch, 1971).

Methaqualone and a few other 2-alkyl-3-aryl-4(3H) quinazolinones were reported to affect the nervous system in a number of ways (Gujaral et al, 1955).

In the words of Brown and Goenechea (1973), "Indeed, of the many congeners of methaqualone which have now been synthesized, only mecloqualone (XII) (Boissier et al, 1958; Dubnick et al, 1969) and its 4'-chloro-derivatives (Hurmer and Vernin, 1967) have therapeutic indices comparable to methaqualone".



XII

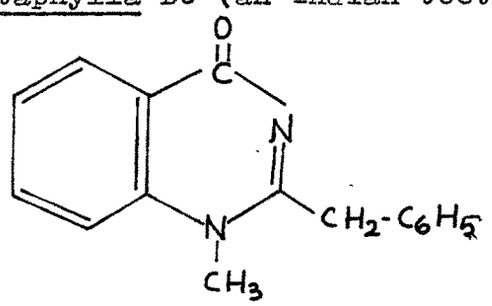
Mecloqualone

Metabolic Effects of Quinazoline Derivatives:

Rather meagre information is available regarding effects of quinazoline derivatives on cell metabolism. It has been reported that methaqualone inhibits the respiration of rat brain homogenate and isolated mitochondria (Parmar and Seth, 1965). Nicotinamide adenine dinucleotide (NAD) dependent oxidation of tricarboxylic acid (TCA) cycle intermediates such as α-ketoglutarate and malate as well as the oxidation of L-glutamate, β-hydroxybutyrate and pyruvate were reported to be inhibited by methaqualone while the oxidation of NAD-independent intermediates of TCA-cycle viz., succinate or NADH, was found to be unaffected (Seth et al, 1964; Parmar and Seth, 1965).

Certain other quinazolone derivatives e.g. 2-methyl-3-(4-acetylbiphenyl)-4-quinazolone was also reported to have similar inhibitory effects on the respiration of rat brain homogenates (Shukla et al, 1967; Parmar et al, 1969). The latter authors reported that the 2,3-disubstituted quinazolones exerted more inhibition than the 3-substituted quinazolones on pyruvate oxidation by rat brain homogenates. Also, attempts were made by them to correlate the inhibition of NAD-dependent oxidations with the anticonvulsant properties of the quinazolone allyl ethers and allyl phenols (Parmar et al, 1971 and 1971a).

Velaso *et al* (1972) have reported that mecloqualone (XII) inhibited the oxygen and glucose uptakes *in vitro* by rat brain tissues. Joshi *et al* (1974) found the inhibition of pyruvate oxidation by rat-brain homogenates by several fluorinated 2-alkyl-3-aryl-quinazolones and corresponding thioquinazolones. Mukherjee and Dey (1970) have reported diminished levels of glutamate and aspartate in mid-brain with simultaneous increase in gamma-amino butyrate levels by glycosin (XIII), a quinazoline alkaloid from *Glycosmis pentaphylla* DC (an Indian toothbrush plant).



XIII

1-methyl-2-(phenylmethyl)-4-(1H) quinazolinone (glycosin)

Several quinazolone salicylhydrazide derivatives were reported to inhibit *in vitro* rat-liver monoamine oxidase (MAO) (Parmar *et al*, 1969a; Gupta *et al*, 1977).

These reports suggest that the 2,3-substituted quinazolones exert their influence on the oxidation reactions of glucose and other metabolites or at the later stages of hydrogen transport to molecular oxygen.

Metabolism of Quinazolinone Derivatives:

Very little information regarding the metabolism of pyridyl substituted quinazolones is available since the metabolism of these compounds are studied only in comparison to that of methaqualone, a tolyl substituted quinazolone. It will be worthwhile, therefore, to describe the available knowledge of metabolism of this compound. A vast literature exists detailing metabolic fate of drugs in general but the exact pathway and mechanism by which this takes place were not known till Brodie and his collaborators (1955) found that the hepatic microsomal enzymes play an important role in their metabolism. According to them, the disposal of foreign compounds by the cell can be accomplished through one or more of the following processes:

(1) Oxidation-reduction (2) hydrolysis (3) lyase type of reactions (4) conjugation with glutathione and subsequent transformation to mercapturic acids (5) ring scission (6) conjugation with glucuronic acid or sulphate or by conversion to methyl or acetyl derivatives (Arnstein et al, 1968).

Metabolism of methaqualone had been studied by several workers. Methaqualone hydrochloride was found to be rapidly absorbed in man (Goenechea et al, 1972). The

drug was found to be present mostly in plasma, bound to the proteins and rarely in blood cells even after administration of large doses (Smart and Brown, 1970; Brown and Goenechea, 1973).

Autoradiographic studies in mice after intravenous administration of 2-^{14}C-methaqualone showed that the drug was rapidly taken up and fixed by fatty tissues, the liver and the brain and excreted relatively slowly (Cohen et al, 1962). It was also reported by Cohen et al (1965) that acid hydrolysable conjugates were present in mouse-urine, but the proportion of unchanged drug was found to be considerably higher than that present in rat or human urine.

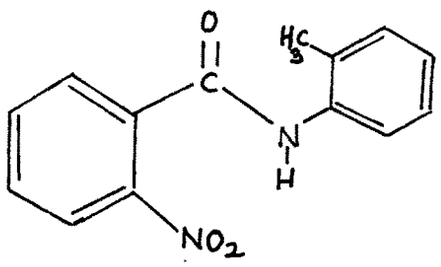
Eberhardt et al (1962) have reported four unidentified urinary metabolites in man after oral administration of methaqualone. Akagi et al (1963, 1963a) have confirmed their findings and have further identified 2-methyl-3-o-(hydroxymethylphenyl)-4(3H) quinazolinone as the major (20%) urinary metabolite in the rabbit, but they failed to detect any glucuronide derivatives in case of man. On the other hand, Beyer and Klinge (1964) inferred after comparison of acid and enzymatic hydrolysis of the urinary metabolites that both glucuronides and sulfates of hydroxy derivatives of methaqualone were also present in the human urine.

Out of twelve or more methaqualone metabolites isolated from the human urine, four of them were found to be hydroxy derivatives with the hydroxy groups located at the 6 position of quinazoline nucleus or at different positions of the tolyl residue (Preuss et al, 1966; Dubnick and Towne, 1972). Other hydroxy derivatives were also identified in the urine of experimental animals (Nowak et al, 1966). The latter investigators were not able to detect unchanged methaqualone in the urine of rabbits, dogs or monkeys, but small quantities in relation to dose, were found in the rat urine. Recently, a catechol derivatives of quinazolinone has been identified (Preuss and Hassler, 1970).

Pretreatment of mice with substances such as carbon tetrachloride (CCl_4) or SKF-525A which are known to cause liver injury produced a potentiation of the effect of methaqualone (Prabhu et al, 1964), while treatment with phenobarbitone well-known to induce the induction of hydroxylating enzyme system of liver increased the excretion of methaqualone and its metabolites in urine (Prabhu and Shah, 1967). Kidneys apparently do not play a significant role in the metabolism of this drug as was evident from their uni- or bilateral nephrectomy experiments (Prabhu et al, 1964).

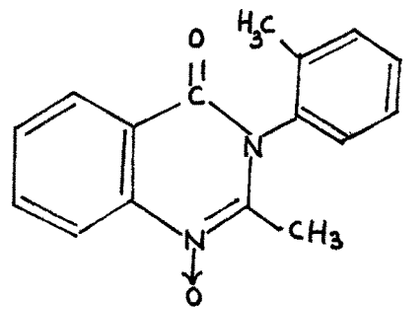
Thus, as in the case of numerous other drugs, liver appears to play an important role in the metabolism of methaqualone also. Unlike in the case of phenobarbitone, a clear-cut evidence for the induction of hepatic microsomal enzymes after administration of methaqualone could not be obtained (Platt and Cockrill, 1969). However, a mixture of methaqualone and diphenhydramine, but not methaqualone alone was reported to induce hepatic drug-metabolizing enzymes in man and in animals (Ballinger et al, 1971; 1972; Stevenson et al, 1972). Mathur et al (1975, 1976) have however, reported that methaqualone alone induced certain microsomal enzymes such as "aniline hydrolase" and "aminopyrine demethylase". According to these authors, phenobarbital was a more potent enzyme-inducer than methaqualone and caused induction of liver enzymes in both young and older animals, while the methaqualone was found to be ineffective in older rats (Mathur et al, 1975,1976).

Cohen et al (1962, 1965) and Nowak et al (1966) have suggested that anthranilic acid, N-acetylanthranilic acid and o-toludine may be the probable metabolites of methaqualone. This was later confirmed by Murata and Yamamoto (1970; 1970a and 1970b). They have isolated a nitrobenzo-o-toluidide (XIV) metabolite from human urine. These authors have also reported an unusual metabolite, 2-methyl-3-o-tolyl-4(3H)-quinazoline-N-Oxide (XV).



XIV

2-nitrobenzo-o-toluidide



XV

2-methyl-3-o-tolyl-4(3H)-quinazoline-N-Oxide

Further studies of Murata and Yamamoto (1970b), on the metabolism of (XV) showed three kinds of metabolites in urine, such as methaqualone, 2-methyl-3-o-(hydroxymethylphenyl)-4(3H) quinazolinone and 2-nitrobenzo-o-toluidide (XIV). Their, *in vitro* experiments, revealed that the N-Oxide (XV) was not oxidized further to the toluidide derivative under conditions of low oxygen tension (air phase) but was reduced to methaqualone. However, the N-Oxide (XV) was converted to, 2-nitrobenzo-o-toluidide (XIV) when oxygen as gas phase was employed.

Stillwell *et al* (1975) have proposed an epoxide-diol pathway of metabolism for methaqualone in rat and man. They have reported seven different dihydrodiol metabolites of methaqualone as minor components in the urine of patients. In the rat, however, after a single acute dose only trace amounts of dihydrodiol metabolite could be detected. Their results suggest

that the epoxidation of methaqualone may represent only a minor pathway in the rat but may be the major one in man (Cf.Booth and Sims, 1974).

The foregoing review reveals the paucity of information with regard to the metabolism of this class of compounds or their effects, on specific metabolic reactions. Though investigations have been carried out in this respect, most of them are merely attempts to correlate the pharmacological properties of these derivatives, with their inhibitory effects on one or two selected enzymatic reactions. Also, no information is available with regard to their effect on, say, carbohydrate metabolism or protein metabolism. Further, the influence of these compounds on many other enzymes have not also been well investigated. The fact that the substituted quinazolinones inhibit respiration in the brain tissue, a glycolysing organ and the report that these substances may be acting as antagonists of NAD (Seth et al, 1964; Parmar and Seth, 1965) have led to the present study. It would also be interesting to study whether these substances influence the metabolic reactions by dint of their reported ability to form complexes with metal ions.

Bacteria was chosen because of the reported inhibition in their growth by quinazoline derivatives

and was employed in this study since glucose is metabolized to a major extent in this organism by the glycolytic pathway.

The present study is thus an attempt to find out whether the pyridyl substituted derivative, SRC-820, inhibits growth and respiration in bacteria and if it affects the carbohydrate metabolism and the re-oxidation of reduced NAD^+. For comparison, other derivatives such as methaqualone, quinazolinone, 3-methyl pyridine and 8-hydroxy quinoline are also employed. The results w are presented in the succeeding pages.