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THE PHARMACOLOGY AND BIOCHEMISTRY OF N-OXIDES

M. H. BICKEL

Medizinisch-chemisches Institut, University of Berne, Berne, Switzerland

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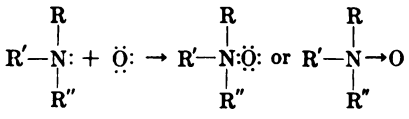
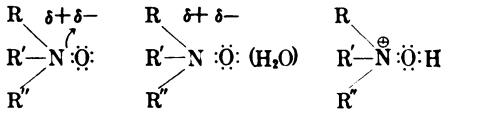
I. INTRODUCTION

In 1894 Dunstan and Goulding discovered the formation of $(CH_3)_3NO$, which they called trimethyloxamine, by the reaction between methyliodide and hydroxylamine. This compound, described in detail in 1899 (55), was the first of a new class of compounds later named amine oxides or N-oxides. The N-O-bond is formed by the lone pair electrons of nitrogen filling the electron gap of an oxygen atom thus forming a semipolar bond or coordinate covalence. This bond is usually indicated by a short arrow:



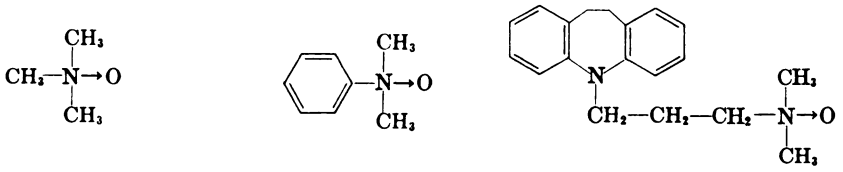
The N-oxides thus are saltlike compounds which are much less basic than the corresponding tertiary amines. Because of the higher electron affinity of oxygen as compared to nitrogen, an electron transfer occurs in the direction of the arrow. This results in a formal charge, a large dipole moment, and a highly

increased polarity. Hydrate and salt forms of N-oxides are also known:



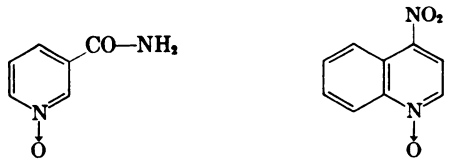
Electron transfer | Hydrate | Salt cation (protonated form)

Some prototypes of aliphatic N-oxides are:



Trimethylamine-N-oxide | Dimethylaniline-N-oxide | Imipramine-N-oxide

In aromatic N-oxides, according to the classification of Ochiai (142), the tertiary amino nitrogen is included in an aromatic heterocyclic ring. Because of resonance stabilization these N-oxides have a more stable N-O-bond; they are less reducible and far less polar than aliphatic N-oxides. Dimethylaniline-N-oxide belongs to the aliphatic group since the lone pair electrons of the oxygen atom are not conjugated with the aromatic π-system. Some prototypes of aromatic N-oxides are:



Nicotinamide-N-oxide | 4-Nitroquinoline-1-oxide

N-Oxides should not be confused with hydroxylamines, which are oxidation products of secondary and primary amines. The reaction leading to the formation of hydroxylamines is sometimes also called N-oxidation. This term is thus used both in its general sense and in the strict sense of N-oxide formation:

Tertiary amine | N-Oxide | Primary or secondary amine | Hydroxylamine derivative

The first papers dealing with the biological significance of N-oxides, *viz.*, their natural occurrence, and their biochemical and pharmacological properties, appeared in 1907. The number of papers published since then shows a remark-

able increase during the last 10 years and has in fact doubled since 1965. Some of the reasons for this increase in interest may be the following: 1) The natural occurrence of N-oxides in plant and animal tissues has posed interesting problems as to the biochemistry and function of these compounds in biological systems. 2) Several N-oxides have become pharmacological agents used in therapy (alkaloids, chemotherapeutics, antibiotics, psychotropic drugs), others are of pronounced toxicological interest (methemoglobin formation, carcinogenesis). 3) N-oxides are metabolites of many tertiary amine drugs and have been postulated as intermediates in N-dealkylation. 4) Certain N-oxides possibly formed in mammalian tissues act as antimetabolites or carcinogens and are postulated as possible inducers of spontaneous cancer.

This review is intended to present and discuss the most important findings published since 1907 and to analyze the abundant data produced since 1960. The scope is restricted to findings pertinent to pharmacology, biochemistry, and related biomedical sciences. The strictly chemical literature on N-oxides has not been included in this work. The literature has been surveyed up to early 1969, but the references given do not provide a complete bibliography on the subject. Some 100 N-oxides have been reported in the biomedical literature; but the emphasis in this review rests on those compounds most frequently dealt with, *viz.*, the N-oxides of trimethylamine, dimethylaniline, 4-nitroquinoline, imipramine, chlorpromazine, nicotinamide, and certain alkaloids. Compounds of pharmacological interest are particularly emphasized.

The previous reviews deserve a particular mention as key references. The biological importance of N-oxides was first reviewed by Culvenor in 1953 (46). In recent years Cheymol *et al.* (39) reviewed the literature on the prototypical trimethylamine-N-oxide. Ochiai's book on *Aromatic Amine Oxides* (142) deals with the chemistry of these compounds; however, it contains a useful chapter on their biological properties. Finally, the topic of "Purine-N-oxides and cancer" was reviewed by Brown (29) in 1968 and hence will not be further discussed in this review. Many references mentioned in these four reviews have been omitted from this work.

II. NATURAL OCCURRENCE OF N-OXIDES

Trimethylamine-N-oxide, the simplest N-oxide, was not only the first to be synthesized, but also the first to be discovered as a constituent of living tissues. The compound was found to occur in muscle of the piked dog fish (*Acanthius vulgaris*) in a concentration of 1 mg/g by Suwa (186) in 1909. In the following decades trimethylamine-oxide was detected in a variety of fishes such as herring (*Clupea harengus*) (155), salmon (17) and other teleosts (93, 138), and selachians (93, 94, 113) and other elasmobranchs (93, 138). Dyer (56) detected trimethylamineoxide in 60 species of fishes and 9 marine invertebrates. He found the highest concentrations in muscle of the elasmobranch fish (0.75 to 2.5 mg/g) but could not detect the substance in fresh-water species. By analyzing the urine of the goosefish (*Lophius piscatorius*), Grollman (81) made the remarkable discovery that 41% of the nitrogen was excreted as trimethylamineoxide. Other

nitrogen-containing excretory products were creatine (28%), amino acids (14%), undetermined compounds (11%), creatinine (4%), and smaller amounts of urea, uric acid, ammonia, and trimethylamine.

Other authors found trimethylamineoxide to occur in species of cephalopods (86, 90), crustaceans (90, 94, 138), lower vertebrates (56, 94), and marine mammals (56, 90). Among mammals the rabbit has been shown to excrete this N-oxide in minor amounts, *viz.*, 0.3 mg/day (16). Figures reported for normal human urine are 60 mg/day, equal to 0.1% of total nitrogen excretion (119), and 2.5 to 5.0 mg/day (184). Trimethylamineoxide has been found in numerous species; the subject has been comprehensively reviewed by Cheymol *et al.* (39).

N-Oxides other than trimethylamineoxide have not yet been unequivocally detected as body constituents of animals, but they frequently occur in plants and microorganisms. In 1915 Polonovski and Nitzberg (159) isolated an alkaloid occurring in addition to eserine in calabar beans. The authors gave the substance the name geneserine and later proved it to be eserine-N-oxide. Its interesting properties led to a promising development in pharmacology and toxicology (see sections III and IV). In *Sophora flavescens* Ochiai and Ito (143) detected oxymatrine, the N-oxide of the alkaloid, matrine. A number of N-oxides of alkaloids occur in association with their corresponding tertiary amines in *Senecio* species, *viz.*, the N-oxides of seneciphylline and platyphylline (6), retronecine (isatinecine) (116), and senecionine (112). An analogous situation has been observed by Fish *et al.* (64) in species of *Piptadenia*, where N,N-dimethyltryptamine and bufotenine were found in association with their N-oxides.

Finally examples of N-oxides found in microorganisms are: iodinine, the pigment of *Chromobacterium iodinum* (42), aspergillic acid, an antibiotic of the mold, *Aspergillus flavus* (207), and myxine, also an antibiotic, from a *Sporangium* species (177). Other N-oxides from plants and microorganisms are mentioned in Ochiai's monograph (142).

About two to three dozen N-oxides are reported in the literature as naturally occurring compounds. Since many of the reports originate from the early decades of this century when analytical methods were poor, errors might have occurred. Formation of artifacts by oxidative attack on a tertiary amine during the process of isolation cannot always be ruled out. Furthermore N-oxides occurring in minute amounts might have escaped detection. In many cases the ability of tissues to synthesize N-oxides has been shown by N-oxidation of potential precursors *in vitro* or *in vivo*: examples are nicotinamide (27, 35–37) and many foreign compounds (see section V). Hence it is probable that many more N-oxides occur naturally than have been demonstrated to the present.

III. BIOCHEMISTRY OF NATURALLY OCCURRING N-OXIDES

A. Trimethylamine-N-oxide

The formation and biochemical fate of trimethylamine-N-oxide has been more intensively studied than that of any other naturally occurring N-oxide. Most investigators (17, 56, 93) agree that this N-oxide is abundant in marine fish but almost completely absent in fresh-water species. Benoit and Norris (17)

observed that the substance is virtually absent in young salmon dwelling in fresh water, whereas considerable amounts are found in the adult animal living in sea water. Transfer of young salmon into sea water does not significantly change the N-oxide level unless they are fed a diet rich in trimethylamineoxide. In man too, a diet of marine fish increases urinary trimethylamineoxide excretion 5 to 25 times (119); the tertiary amine shows only a minor increase. These observations are strongly in favor of the view that in the salmon and man trimethylamineoxide is of dietary rather than of endogenous origin. A more recent study in spiny dogfish (*Squalus acanthias*) (78) disclosed a constant plasma level of this N-oxide even during starvation; this was interpreted as a slow release from a pool in muscle and a low rate of excretion.

Another line of research has dealt with possible precursors of trimethylamineoxide. Early investigations showed that in animals the compound is associated with betaine (86, 186). Evidence indicates that choline (16, 50, 94, 119, 139) and possibly lecithin (16, 94) and carnitine (184) are precursors. The transformation of choline into trimethylamineoxide seems to be at least partly dependent on the action of enteral bacteria (50, 94, 139). The question as to whether animal trimethylamineoxide is of exogenous, endogenous, or bacterial origin has not yet been solved.

Considerable N-oxidizing activity has been demonstrated in dog (94), rabbit (47, 94), guinea pig and rat (132), a large number of other vertebrates (12), and man (119) after administration of trimethylamine. This activity in the rabbit resides mainly in liver, lung, and kidney (12). Baker and Chaykin (10, 11) proved that the N-oxidation of trimethylamine is dependent on a mixed-function oxidase of the drug-metabolizing enzyme system of liver microsomes. The authors incubated the amine with hog liver microsomes, NADPH, and $^{18}O_2$ and isolated trimethylamine-^{18}O-oxide, NADP, and $H_2{}^{18}O$. This reaction was not inhibited by cyanide.

As early as 1909, Suwa (187) observed that there is also a reduction of trimethylamineoxide to the tertiary amine by bacteria, and by the rabbit. The formation of foul-smelling trimethylamine in spoilage of fish has also been ascribed to bacterial N-oxide reduction (120). Reduction was detected *in vivo* in rats (139), rabbits (47), and man (119). In homogenates of the parenchymatous organs of rats and rabbits, 100% of added N-oxide was reduced to trimethylamine (132). This reaction has been less studied than the N-oxidation of trimethylamine. The most informative paper was written by Ackermann *et al.* (2) more than 40 years ago. According to these authors trimethylamineoxide is reduced by mammalian liver *in vitro* even after boiling; presumably this was a nonenzymatic reaction. Furthermore they were able to reduce this N-oxide by ferrous ions, cysteine, or reduced glutathione. Later authors confirmed these findings except that glutathione or ascorbic acid could not be oxidized by the N-oxide (157, 203) and that ferrous ions could be replaced by hemoglobin (203). The finding of simultaneous N-oxidation and N-oxide reduction obviously complicates the question of the origin of these compounds, raising the new question as to whether trimethylamine or its N-oxide is the primary product of biosynthesis.

The high trimethylamineoxide content of many marine fish has stimulated speculations on the physiological function of this compound. One of the starting points of these speculations was Grollman's finding (81) that the goosefish excretes more than one third of its nitrogen as trimethylamineoxide. Hoppe-Seyler (93) considered this substance to be an end-product of nitrogen metabolism, far less toxic than ammonia and useful in the maintenance of the osmotic balance. This hypothesis is hardly worth considering, however, since the blood level of trimethylamineoxide is very low as compared to its urinary excretion, and since it is also excreted by the gills (Baldwin (13)).

The discovery of a biological reduction of trimethylamineoxide by Ackermann *et al.* (2) suggested a possible function as an oxidant or hydrogen acceptor particularly for sulfhydryl groups. Since N-oxidation of trimethylamine occurs in addition to N-oxide reduction, the suggestion has widened to a possible function as regulating system for redox processes. Indeed the quotient TMA/TMA-N-oxide in urine has been introduced and postulated as an index of oxidation processes in the body, such as the ratio of oxidative metabolism to glycolysis (89). The quotient has also been said to be significantly changed in cancer patients (89) and in persons with hyperthyroidism (184).

In spite of much work performed over more than half a century the ideas on the physiological function of trimethylamineoxide are as vague as the clues to its origin. More detailed references are given in the review of Cheymol *et al.* (39).

B. Other N-oxides

Other than trimethylamineoxide the most thoroughly investigated compound of this class is nicotinamide-N-oxide. Its natural occurrence has not yet been proved but is very probable since administered nicotinamide is easily N-oxidized in rats (35) and mice (27, 36, 37, 107); this results in the formation of the N-oxide and other metabolites, detected in urine and tissues. Chaykin's group, to which we owe most of our knowledge of nicotinamide-N-oxide, found that the N-oxidation of nicotinamide, like that of trimethylamine, is due to the action of the liver microsomal enzyme system dependent on NADPH and O_2, and that extrahepatic tissues show practically no activity (107).

N-Oxide reduction of nicotinamide-N-oxide has also been demonstrated in liver homogenate of mice (27) and hogs (35). This reaction has received more attention and seems to depend on mechanisms other than those involved in the corresponding reduction of trimethylamineoxide. According to the investigations with hog liver homogenates by the Chaykin group, an enzymatic reaction dependent on NADH is involved; the enzyme has been purified (134). Furthermore the authors were able to show that xanthine oxidase of either the liver or milk, with its usual electron donors, xanthine and hypoxanthine, can reduce nicotinamide-N-oxide, and that this reaction is not dependent on oxygen but is inhibited by cyanide (135). Direct oxygen transfer from the N-oxide to xanthine has also been demonstrated (136).

The reduction of the derivative, nicotinic acid-N-oxide, has been detected by Tatsumi and Kanamitsu in the rat *in vivo* (192) and by microorganisms (194)

such as *Escherichia coli* (195). In liver perfusion experiments (193) the authors detected little activity in the rabbit and none in the rat.

Again the occurrence of both N-oxidation and N-oxide reduction suggests that these N-oxides may be biological oxidants. This has been emphasized by Chaykin *et al.* (35, 136) for nicotinamide-N-oxide and heterocyclic N-oxides in general. A similar situation may be found in the case of purine-N-oxides. They have not yet been found to occur naturally, but they are antimetabolites or oncogenic agents; this suggests that such derivatives may play a role in the origin of spontaneous cancers. Brown (29) has expertly treated the field of purine-N-oxides and cancer, and has cited the original literature in his review.

N-Oxides occurring in plants have been much less investigated with respect to their phytochemical behavior. One of the remarkable exceptions is the studies carried out by Areshkina (6–8) on the *Senecio* alkaloids, seneciphylline and platyphylline, and their respective N-oxides. According to this author both the tertiary amines and their N-oxides are present in these plants, the ratio varying according to the stage of growth. The N-oxide form predominates in the growing state and reaches a maximum before flowering, whereas the tertiary amine form predominates in the resting state of seasonal degeneration. These N-oxides oxidize ascorbic acid and other substrates *in vitro* and hence may be constituents of a redox system acting on other plant metabolites. A discussion of this interesting contribution to plant biochemistry was included in Culvenor's review (46). Wenkert (206) proposed that N-oxides formed by plant oxidases are intermediary compounds in the pathway leading from amino acids to alkaloids, which represent a higher oxidation level.

Fish *et al.* (64) made the interesting discovery that species of *Piptadenium* contain not only N,N-dimethyltryptamine but also its N-oxide, as well as bufotenine (N,N-dimethylserotonin) and its N-oxide. The distribution pattern suggests the following biosynthetic pathway: dimethyltryptamine → bufotenine → bufotenine-N-oxide. These and certain other alkaloid-N-oxides of pharmacological interest have been studied with respect to their behavior in the mammalian body (see sections IV to VI).

IV. PHARMACOLOGY AND TOXICOLOGY OF N-OXIDES

A. Alkaloid N-oxides

The discovery of geneserine (eserine N-oxide) and the study of many other alkaloid-N-oxides by Polonovski and Polonovski in the nineteen twenties initiated an interesting line of research and of potential pharmacotherapeutic applications. Geneserine and the N-oxides of tropane and strychnos alkaloids were reported to exert an action similar to that of their respective tertiary amines but without their toxicity (160). The authors postulated a continuous reduction of the N-oxides in the body and thus a slow release of active tertiary amine alkaloid (156, 160). The interesting principle of retained pharmacological activity and strongly decreased toxicity was also reported by these authors for the N-oxides of strychnine, atropine, hyoscyamine, scopolamine, and morphine, most of which have been prepared synthetically (156). Other synthetic N-oxides

investigated were prepared from cinchonine, acetylmorphine, aconitine, arecoline (161), nicotine, granatane (164), N-methylgranatoline, tropinone, scopoline, and emetine (163). The decreased toxicity of the N-oxides was explained by their water solubility and increased excretion. The highlight of these findings was clearly the higher therapeutic index of these compounds and therefore the possibility of a broader clinical use of many alkaloids. This became particularly obvious in the case of morphine-N-oxide, which was reported (158) to have the same action as the parent compound, one-fourth of its activity, low toxicity, and no habit-forming properties. It was even proposed for the treatment of addicts. These optimistic reports did not stand the test of time. Indeed investigators outside the Polonovski group reported that the N-oxides of morphine, as well as of thebaine, codeine, ethylmorphine (72), and narcotine (53) are practically inactive. In a recent study the potency of morphine-N-oxide was one-tenth to one-ninetieth that of the parent alkaloid, depending on the mode of administration (60). The promising era of alkaloid N-oxides has thus become a historical interlude.

N,N-Dimethyltryptamine and bufotenine, detected in association with their respective N-oxides in *Piptadenia* seeds (64), are known as psychotomimetic agents, but no indication is given whether the N-oxides share this property. The fact that 5-hydroxytryptamine does not appreciably cross the blood brain barrier suggests that the more polar bufotenine-N-oxide would not share the psychotomimetic action of its parent compound. Tumor-inhibitory action has been reported for both senecionine and its N-oxide (112).

None of the studies reporting on pharmacologically active alkaloid N-oxides include useful data on the fate of these compounds in the body. Thus it is not known whether these N-oxides are active *per se* or only because they are reduced to their tertiary amines.

B. Naturally occurring and antibacterial N-oxides

Most of the alkaloid N-oxides mentioned above have been prepared synthetically and are not reported to occur in plants. Among the naturally occurring N-oxides, the diuretic and hypertensive properties of trimethylamine-N-oxide were reported by Cheymol *et al.* (39), but no therapeutic use has been made of this substance. Its LD50 in mice is 2850 mg/kg as compared to 90 mg/kg with the parent amine. The N-oxide reduces the toxicity of trimethylamine in a noncompetitive manner when injected simultaneously (48).

The abortive attempt to introduce alkaloid N-oxides into therapy was followed by a more successful development around 1940. The starting point was the discovery that the naturally occurring N-oxide, iodinine (42), is antibacterial. Subsequently the antibiotic aspergillic acid (207) was found to inhibit certain Gram-positive and Gram-negative bacteria as well as tubercle bacilli; its toxicity, however, was prohibitive for clinical use. These two naturally occurring prototypes opened a search for antibacterial compounds. N-Oxides were isolated from other fungi, analogues of iodinine (127) were prepared, and further synthetic work led to a number of useful chemotherapeutic compounds, *e.g.*,

N-oxides of benzotriazines (208), quinoxalines (148), and pyridylalanine (185). Several aromatic N-oxides with antimicrobial activity are considered in Ochiai's book (142, chapter 9).

C. 4-Nitroquinoline-N-oxide and other carcinogens

One of the most interesting synthetic N-oxides, investigated mainly by Japanese workers, is the carcinogen, 4-nitroquinoline-N-oxide. It was first reported to possess antibacterial (5) and antifungal (144, 145) properties; later its carcinostatic and carcinogenic (57) properties were discovered. The substance has indeed become known as a model carcinogenic compound.

Structure-activity relationships have been well studied with this compound and its analogues. Arai and Nakayama (5), the authors of the first publication, reported the N-oxides of quinoline and pyridine to be more potent carcinogens than the tertiary bases, and analogues with the nitro- or amino group in position 4 to be the most potent. The observation that the N-oxide group is essential for activity has been confirmed by several authors (102, 103, 144). Furthermore the carcinogenic activity has been shown to be mediated by an active metabolite, 4-hydroxylaminoquinoline-N-oxide (102, 103, 122, 176), probably formed by an enzyme, present in rat liver (188), which reduces the nitro group but not the N-oxide. Reduction of the latter by cysteine or reduced glutathione inhibits the carcinogenic action of 4-nitroquinoline-N-oxide (145). Among a large number of 4-nitroquinoline derivatives only those that were metabolized to 4-hydroxyaminoquinoline-N-oxide exhibited carcinogenic action (102).

In recent years 4-nitroquinoline-N-oxide has been studied with respect to its interaction with nucleic acids (4, 108, 121, 147, 150, 190) and proteins or amino acids (98, 146). These properties are possibly related to the mechanism of its carcinogenic action.

4-Nitroquinoline-N-oxide is not the only carcinogenic agent among N-oxides. Its 6-chloro derivative is even more potent (191). Other carcinogenic agents are the N-oxides of 2-methoxyphenazine (58) and of nitrobenzofuroxan, the latter also inhibiting RNA synthesis (74). According to an interesting report (3) the N-oxide of nitrogen mustard has twice the curative effect and one-tenth the toxicity of its carcinostatic parent compound. This finding, strongly reminiscent of the "alkaloid N-oxide era," has also been contradicted (57).

D. Psychotropic N-oxides

There are pharmacologically active N-oxides in all major subgroups of psychotropic drugs: tranquilizers, neuroleptics, and thymoleptics. One example is the tranquilizer, chlordiazepoxide, today one of the most frequently used drugs in psychiatric and general medicine. The corresponding benzodiazepine derivative without oxygen at the nitrogen atom is also active, but in some tests less so than chlordiazepoxide (92). One of its metabolites is a lactam which retains both N-oxide group and psychotropic activity (170).

The neuroleptic drug chlorpromazine is transformed into numerous metabolites, including the N-oxide, which has been discovered as a minor excretion

product in the urine of patients (69). It is less potent than the parent compound or desmethylchlorpromazine, but more potent than chlorpromazine sulfoxide (167). The same authors found that the N-oxide is the only major chlorpromazine metabolite showing a lag in onset of action. The lag suggests that it has no activity *per se*, but is transformed to an active metabolite.

Fishman and Goldenberg, the discoverers of chlorpromazine-N-oxide (69), were also the first ones to isolate imipramine-N-oxide from the urine of patients receiving the parent thymoleptic drug (68). The investigators who compared the N-oxide to imipramine found similar but less pronounced central and peripheral actions (198), absence of ECG changes or fall in blood pressure, and equal or decreased acute toxicity (109). Both compounds lack teratogenic effects in rabbits (114). In 1963 Faurbye *et al.* published favorable results concerning a clinical trial of imipramine-N-oxide in depressed patients (59). Other clinical investigators (30, 79, 85, 109), reporting on a total of 295 patients, agreed that the action of the N-oxide is similar to that of imipramine and other thymoleptics and involves fewer side-effects. In Denmark imipramine-N-oxide has recently been introduced into therapy.

E. Miscellaneous N-oxides

An interesting compound used for inhibition and prophylaxis of silicosis is the water-soluble high molecular weight polymer, polyvinylpyridine-N-oxide (117, 168, 172, 183). In minute amounts the compound protects against quartz-induced hemolysis and fibrosis, and increases collagen synthesis. Distribution in the body (118) and at a subcellular level (62, 73) has been studied in an attempt to elucidate the mechanism of action.

The synthetic compounds, N,N-dimethylaniline and its N-oxide, have been studied primarily with respect to their metabolic fate. This N-oxide has received attention as a possible carrier of the methemoglobin-forming properties of dimethylaniline; indeed the N-oxide forms methemoglobin when incubated with hemoglobin aerobically (105). Convincing experiments *in vivo* (106, 201) showed that the N-oxide is not a major cause of the methemoglobinemia and can be ruled out as a toxic metabolite. On the other hand the N-oxide of the aromatic amine, N,N-4-dimethylaminoazobenzene is more active in forming methemoglobin. Kiese (104) reviewed N-oxides of aromatic amines as possible methemoglobin-forming metabolites. Since dimethylaminoazobenzene (butter yellow, formerly used as food coloring) is carcinogenic, its metabolically formed N-oxide has been included in structure-activity studies (99) and has been found to produce malignant hepatomas in rats (197).

The insecticide Schradan (octamethylpyrophosphoramide) is an anticholinesterase *in vivo*, but is virtually inactive *in vitro*. The substance undergoes a metabolic activation in mammalian liver, and in insect and plant tissue. The active metabolite has been identified as Schradan-mono-N-oxide, a cholinesterase inhibitor as potent as DFP (33). Schradan-N-oxide has also been obtained by oxidation of Schradan with permanganate. The O-methyl-, methylol-, and demethylated (heptamethyl-) derivatives have been postulated as secondary

TABLE 1

N-oxides more potent than their corresponding tertiary amines

N-oxide	Action	Reference
4-Aminoquinoline-N-oxide	Carcinogen	5
Chlordiazepoxide	Tranquilizer	92
Diallylmelamine-N-oxide	Vasodilator	215
p-Diazine-di-N-oxide	Antibacterial	127
Nitrogen mustard-N-oxide	Oncostatic	3
4-Nitroquinoline-N-oxide	Carcinogen	5, 144, 103
Pyridylalanine-N-oxide	Antibacterial	185
Schradan-N-oxide	Insecticide	33, 199

metabolites (199). On fractionating the oxidation products obtained by exposing Schradan to neutral permanganate, O'Brien and Spencer (141) identified one of the compounds as the active metabolite. They interpreted this compound as the monomethylol derivative rather than the N-oxide (182). A critical examination of the reports does not yield unequivocal evidence in favor of either the N-oxide or methylol theory.

Another drug forming an active N-oxide metabolite is the vasodilator diallylmelamine. Its N-oxide, formed in rats and dogs, is 20 times more potent than the parent compound (215). A list of N-oxides reported to be more potent than their corresponding tertiary amines is given in table 1.

As has already been mentioned, certain tertiary amine drugs form N-oxides less potent than their parent compounds. Among these is chlorcyclizine-N-oxide, whose antihistaminic activity is about one-fourth that of the unchanged drug (111). Guanethidine forms an N-oxide with one-tenth of the antihypertensive activity (131). The central cholinergic action of tremorine is dependent on the formation of oxotremorine by C-oxidation. It also forms an N-oxide which is pharmacologically similar to the parent compound (40). To further demonstrate the diversity in pharmacological action of N-oxides mention is made of the diuretic 8-chloroalloxazine-5,10-dioxide (151), the radioprotective compound quinoxaline-1,4-di-N-oxide (82), and the toxic irritant trifluoroamine oxide, a carbon-free N-oxide (38).

V. N-OXIDES AS METABOLITES OF DRUGS

A. Drugs forming N-oxides

In mammals and mammalian tissues many tertiary amine drugs are metabolized to N-oxides. In most cases other metabolites such as N-dealkylated derivatives are also formed. Well known drugs forming N-oxides are listed in table 2; the list is not meant to be complete. In many drug metabolism studies the methods used would not have been adequate for the detection of N-oxides. Since N-oxides as a rule have been found to be minor metabolites, lack of sensitivity is another factor. Thus it is likely that N-oxides are formed in many more instances. If they occur in the body only in minute amounts this can be due to

TABLE 2

Tertiary amine drugs forming N-oxides in animal tissues

Tertiary amine	Reference
3-Acetylpyridine	137
Chlorcyclizine	31, 111, 166
Chlorpromazine	14, 15, 44, 69, 70, 83, 84, 100, 165
Cinnarizine	181
Diallylmelamine	215
Dimethacrine	171
Dimethylaminoazobenzene	200–202
Dimethylaniline	1, 9, 88, 91, 126, 153, 180, 200, 201, 211–214
Dimethyltryptamine	67, 189
Diphenhydramine	52
Guanethidine	131
Hydroxyzine	43
Imipramine	18, 19, 21, 22, 24, 41, 68, 87, 110
Morphine	209
Nicotinamide	35
Nicotine	149
Nikethamide	34
Promazine	77
1-Propoxyphene	130
Schradan	33, 199
Tremorine	40
Trimethylamine	11, 12, 47, 132, 133

either a very low rate of formation or a rapid rate of further metabolic conversion. In a few instances such as methixene (115), isoniazid (152), and diazepam (170, 174) the formation of N-oxides could not be detected although adequate and sensitive methods were used. In other cases, *e.g.*, dimethylaniline (95, 96) or dimethylaminoazobenzene (204), negative results have been reported contrary to the findings of many other authors. In these cases differences in the biological systems or species used may have been involved.

B. Mechanism of N-oxide formation

The formation of N-oxides from tertiary amines has been investigated by many authors. In many of these investigations amines were incubated *in vitro* with tissue preparations to evaluate localization of enzymatic activity in organs and subcellular fractions as well as cofactor requirements. Virtually all of these investigations led to the conclusion that the metabolic formation of N-oxides occurs in the microsomal fraction of liver under aerobic conditions and requires NADPH. This result was obtained with many tertiary amines such as: dimethyltryptamine (189), dimethylaniline (9, 91, 126, 153, 179, 210, 212–214), nicotine (149), propoxyphene (130), imipramine (18, 19, 24), dimethylaminoazobenzene (200, 202), and chlorpromazine (14, 15, 44, 83, 84, 100). The same is true with N-oxide formation from the naturally occurring tertiary amines trimethylamine

(11, 12) and nicotinamide (107) (see section III). In addition, trimethylamine incubated in the presence of $^{18}O_2$ formed trimethylamine-N-^{18} oxide and $H_2{}^{18}O$ (11), as expected with a mixed-function oxidase.

The action of a microsomal enzyme requiring NADPH and molecular oxygen suggests the involvement of the hepatomicrosomal electron transport chain that oxidizes drugs, steroids, and other lipophilic compounds. It is therefore interesting to consider whether N-oxide formation is characteristic of this enzyme system, in other respects *viz.*, localization in liver, ontogenic development, roles of cytochrome P-450 and NADPH-cytochrome c reductase, inducibility by drugs like phenobarbital, and inhibition by CO, SKF 525-A (β-diethylaminoethyl-diphenylpropylacetate), *p*-chloromercuribenzoate, and cytochrome c, but not by cyanide. In an investigation of trimethylamine, the rate of N-oxide formation by microsomes from liver, lung, and kidney showed a ratio of 26:7:4 and was negligible in muscle, brain, and ovaries (12). Similar results obtained with dimethylaniline (124) and imipramine (26) indicate a predominantly hepatic localization with minor activities in lung and kidney. The few studies done on induction of tertiary amine N-oxidase suggest inducibility of N-oxide formation from dimethylaniline by barbital (124). Ontogenic development of the enzyme activity has been observed by comparing the livers of adult bullfrogs to those of their tadpoles (133). N-Oxidation of trimethylamine (11) and imipramine (26) is not inhibited by cyanide. Imipramine N-oxidation is inhibited by *p*-chlormercuribenzoate (26). These results support the suggestion that N-oxidation, like hydroxylation or N-demethylation, is catalyzed by the microsomal electron transport chain requiring NADPH and oxygen, but several further results cast doubt on the assumption that N-oxide formation uses the identical microsomal system including NADPH-cytochrome c reductase and cytochrome P-450. The well known inhibitors of this enzyme system, CO and SKF 525-A, are without effect on N-oxidation of dimethylaniline (126, 179, 213), chlorpromazine (44, 83), and imipramine (26), and this effect would be expected if P-450 were involved in microsomal formation of N-oxide. Furthermore dimethylaniline is still N-oxidized by cholate-treated or sonificated microsomes (9, 212); in other words, this process, contrary to oxidations dependent on P-450, does not depend on the integrity of the microsomal membrane structure. In the case of imipramine, N-oxide formation has proved to be far more sensitive to a decrease of pO_2 than the corresponding N-demethylation or aromatic hydroxylation (26). Furthermore the cofactor requirement is not identical for these reactions. Ziegler's group found dimethylaniline N-oxidase to depend on the presence of flavine-adenine nucleotide (FAD) (153) and subsequently purified the enzyme 1000-fold by sonification, treatment with detergents, ultracentrifugation, and salt precipitation (83, 100, 210, 214). This enzyme preparation also N-oxidized chlorpromazine (83, 100). It contained a flavoprotein that is not identical to NADPH-cytochrome c reductase (210, 214). The results reported thus suggest that, contrary to common assumption, tertiary amine N-oxidation does not strictly follow the route of microsomal oxidative drug metabolism. However, the process is also dependent on liver microsomal enzymes, NADPH, and oxygen.

Species differences in N-oxide formation *in vitro* have been described for trimethylamine (12, 132), dimethylaniline (124, 212), chlorcyclizine (111), imipramine (18), and chlorpromazine (44, 70, 84). Rates of N-oxide formation have been evaluated for dimethylaniline (124, 153, 212), propoxyphene (130), imipramine (18, 19, 24), and chlorpromazine (14, 15, 44, 100). Since the systems used are not identical the rates cannot be compared. However, according to most authors, when multiple pathways of the metabolism of a tertiary amine are present, formation of N-oxide is usually a minor pathway. For chlorpromazine (15) and other phenothiazines (14) *in vitro*, however, Beckett *et al.* concluded that N-oxides are the major metabolites. Whether the use of inadequate methods for quantitative determination by other authors is the sole reason for this discrepancy is not yet clear. Metabolism of drugs by multiple metabolic pathways may result in the formation of several metabolites containing N-oxide groups. Thus chlorpromazine yields its N-oxide (7%), its N-oxide-sulfoxide (1.2%), and 7-hydroxy-chlorpromazine-N-oxide (0.2%) (44). It is obvious that discussion of the quantitative aspects of N-oxide formation requires a knowledge of the further transformations of N-oxides (section VI).

C. Distribution and excretion of N-oxides

Very little data have been reported on distribution, excretion, or other translocation properties of N-oxides. An average of 1% of the dose was determined as urinary N-oxides after the administration of chlorpromazine (69), promazine (77), imipramine (41, 68, 87, 110), or chlorcyclizine (111) to patients. After administration of the last drug to rats, 1% N-oxide is excreted by males and 4% by females (31, 111). These findings indicate a partial transformation of tertiary amines into N-oxides, but the amount measured is the net result of formation, metabolism, and renal clearance of the N-oxides.

Detection of N-oxides in tissues was attempted after administration of trimethylamine (132), chlorcyclizine (111, 166), chlorpromazine (71), imipramine (22, 25), and dimethacrine (171) to laboratory animals or people. In all these instances the N-oxides were detected in urine but not in tissues. Rapid urinary excretion was proposed as an explanation of the pharmacological inactivity of a number of alkaloid-N-oxides as early as 1910 (72). Through these experiments little information could be gained on the distribution of the N-oxides, since the precursor amines have been administered and the resulting N-oxides are usually minor metabolites. Indeed after direct administration, poly-2-vinylpyridine-N-oxide (118) and chlorcyclizine-N-oxide (111) can be detected in tissues. The latter was detected when given intraperitoneally but not when given by mouth, although at least part of the drug must also have been absorbed after oral administration. In rats and guinea pigs treated with imipramine the N-oxide is present in plasma, bile, intestinal contents, feces and urine, but not in tissues (22, 23, 25); whereas after direct administration of imipramine-N-oxide the compound could also be detected in liver, lung, kidney and other tissues, but not in brain (23, 26).

These experiments demonstrate the striking differences between tertiary

amines and their corresponding N-oxides with regard to distribution and excretion. These differences are obviously caused by physicochemical differences between these types of compound, *e.g.*, the markedly increased polarity of N-oxides as compared to their parent tertiary amines. In a recent study (23) the physicochemical and the resulting pharmacokinetic properties of imipramine, its N-oxide, and other imipramine metabolites have been investigated. The transformation of imipramine to its N-oxide leads to a remarkable increase in polarity, as can be demonstrated by the partition values in various organic solvents or by the buccal absorption test. In addition the pK_a shifts from 8.0 to 4.7, and this shift explains why the passage of imipramine-N-oxide into nonpolar solvents or across biological membranes is not pH-dependent, unlike that of the tertiary amine. These physicochemical properties of N-oxides seem to be responsible for their physiological distribution pattern and rapid renal excretion. Furthermore they are important for the development of effective extraction methods for N-oxides (68, 80, 205). Neglect of solubility or partition values of N-oxides may have been the cause for the lack of detection or for underestimation of N-oxides in certain cases. Finally the relatively high polarity of N-oxides may decrease or prevent their access to certain enzymes, such as the membrane-bound microsomal electron transport chain, and this would influence their further metabolism.

VI. METABOLISM OF N-OXIDE DRUGS

A. Reduction

N-Oxides are easily reduced to their corresponding tertiary amines by chemical means. A paper written in 1910 (72) discarded the possibility of a reduction *in vivo* of alkaloid N oxides since these compounds were found to be pharmacologically inactive, in contrast to the alkaloids. Subsequently, Polonovski (156),

TABLE 3

N-oxides undergoing biological N-oxide reduction

N-oxide	Reference
Chlorcyclizine-N-oxide	111
Chlordiazepoxide metabolite	175
Chlorpromazine-N-oxide	44
Diallylmelamine-N-oxide	215
Dimethylaminoazobenzene-N-oxide	196, 201, 202
Dimethylaniline-N-oxide	76, 201
Dimethyltyrosine-N-oxide	67
Imipramine-N-oxide	21, 24, 25
Nicotinamide-N-oxide	27, 35, 134-136, 192-195
Purine bases, N-oxides of	54
Pyridinealanine-N-oxide	185
Pyridine-N-oxide	123
Tremorine-N-oxide	40
Trimethylamine-N-oxide	2, 47, 132, 203

working with apparently active alkaloid-N-oxides, postulated a slow release of tertiary amine by reduction of the N-oxide. In the meantime N-oxide reduction has been observed with a number of substrates, in several species and systems; a list is given in table 3. Data vary between 100% reductive conversion (132) and mere trace reactions. In the case of the N-oxide drug chlordiazepoxide, only one minor metabolite loses its N-oxide group by reduction (175).

The reduction of trimethylamine-N-oxide by mammalian liver preparations was described as early as 1927 (2). The reaction is heat-stable and could be produced also by ferrous ions, cysteine, or reduced glutathione. Another report confirmed the nonenzymatic nature of this reaction (203). Dimethylaminoazobenzene-N-oxide is reduced to the tertiary amine by ferrous ions, hemoglobin, methemoglobin, or red cells, or by liver microsomes even in the absence of NADPH (121, 196, 202). The fact that during the first 30 seconds, 8% of the substrate was reduced by ionized iron and 50% by heme-complexed iron suggests a catalytic reaction.

In liver preparations (44) chlorpromazine-N-oxide was reduced at the rate of 75% in whole homogenate, 35% in 10,000 × *g* supernatant, and 30% in microsomes. In control incubations with boiled preparations the corresponding figures were 55, 10, and 5%. Hence there is an extensive nonenzymatic and in addition a (possibly enzymatic) NADPH-independent N-oxide reduction. The activity distribution suggests that at least one half of the activity may be localized in extramicrosomal compartments. Furthermore, reduction of chlorpromazine-N-oxide is not inhibited by SKF 525-A and is higher in biopsies of human liver than in rat liver. The reaction can also be produced by ferrous ions and EDTA but not by ferric ions. In contrast to N-oxide reduction no sulfoxide reduction has been observed.

Extensive reduction of imipramine-N-oxide has been observed in guinea pigs and rats *in vivo* (21, 25), and the reaction has been studied in more detail *in vitro* (24). Thus imipramine-N-oxide is reduced by various rat tissue homogenates or blood in the absence of any added cofactors. The reaction is increased by absence of oxygen and is only partially heat labile. In blood the N-oxide reduction, which can be traced to the hemoglobin fraction, occurs within minutes or seconds. In blood-free liver the reaction proceeds after an initial jump; it does not occur in the dialyzable fraction of homogenates. These observations suggest a heme catalysis in blood and both catalytic and enzymatic processes in liver. The reaction can also be simulated by high concentrations of ferrous ions but not ferric or other heavy metal ions; EDTA enhances the Fe^{++}-induced catalysis. Further studies (26) have shown that the activity distribution among whole homogenate, 9000 × *g* supernatant, and microsomes is almost identical with that indicated above for chlorpromazine-N-oxide (44); part of the activity is localized in the nuclear fraction. Reduction of imipramine-N-oxide is inhibited by O_2 but is not influenced by CO or SKF 525-A. In the case of hemoglobin-catalyzed reduction, considerable amounts of methemoglobin are formed *in vitro*. Liver perfusion experiments in the presence and absence of red cells in the perfusion medium showed that about 75% of added imipramine-N-oxide was

reduced by the liver and an additional 20% by the red cells. In totally hepatectomized rats reduction of this N-oxide was only about 5 to 10% lower than in sham operated controls. These experiments demonstrate the importance of extrahepatic tissues and blood in the reduction of N-oxides *in vivo*.

Reduction of nicotinamide-N-oxide to nicotinamide has been observed with hog (35) and mouse liver homogenate (27), rabbit liver perfusion (193), intact rat (192), and microorganisms (194, 195); boiled mouse liver homogenate was inactive. The enzymatic basis of N-oxide reduction has been most extensively investigated with this substrate by Chaykin *et al.* (134–136). The authors were able to purify the enzyme from hog liver, to characterize it as a metalloflavoprotein, and to show its dependence on NADH and possibly other cytoplasmic cofactors (134). In addition they showed that nicotinamide-N-oxide was reduced by xanthine oxidase from liver or milk with xanthine as electron donor (135); the reaction was inhibited by cyanide and partially inhibited by oxygen. Finally, experiments with ^{18}O compounds in the presence of xanthine oxidase revealed the possibility of a direct oxygen transfer from nicotinamide-N-oxide to xanthine, resulting in the formation of uric acid. However, the N-oxide also acted as an electron acceptor (136). The authors raised the possibility that heterocyclic N-oxides may be capable of acting as general biological oxygenating agents.

In spite of the clearcut results obtained in the case of nicotinamide-N-oxide, we are still far from a general explanation of the mechanism of N-oxide reduction. A comparison of the data summarized above shows serious discrepancies: the reaction is reported to be enzymatic, nonenzymatic, or both; heat-labile or heat-stable; dependent on or not connected with xanthine oxidase; or dependent on various cofactors. Much additional work will be required to trace these discrepancies to methodological differences or else to specific properties of N-oxide substrates or types of substrates. The multitude of reducing systems in a higher organism is likely to complicate the task.

One of the interesting aspects of N-oxide reduction is the fate of the oxygen atom. Both electron and oxygen transfer have been established in the case of nicotinamide-N-oxide. A recent report states that dimethylaniline-N-oxide can replace O_2 in some NADPH-dependent microsomal oxidations (173). In the course of the reduction of imipramine-N-oxide by ferrous ions, the formation of neither molecular oxygen nor ferric ions could be detected (26). Some investigators have discussed the possibility of a rearrangement of aromatic N-oxides into aromatic aminophenols (88, 95–97, 189).

Results obtained with the N-oxides of chlorpromazine and imipramine suggest a dual subcellular localization of the N-oxide-reducing activity in rat liver. A similar distribution has recently been observed for sulfite oxidase (EC 1.8.3.1.) (101).

An interesting aspect is that in many cases (compare tables 2 and 3) a simultaneous tertiary amine N-oxidation and N-oxide reduction can occur. Thus the net amount of one product need not be representative for one reaction. There is no doubt that the systems catalyzing the two processes are profoundly different; this aspect will be discussed in a wider context in section VI C.

B. Dealkylation

Dealkylation of N-oxides, resulting in the production of the secondary amine and an equivalent amount of alkylaldehyde, can be achieved by a reaction with acetic anhydride, the so-called Polonovski reaction (45, 140, 162). Demethylation of trimethylamine-N-oxide occurs in the presence of iron or iron complexes (61, 203). Fish *et al.* (65, 66) described demethylation of N,N-dimethyltryptamine-N-oxide by ferric ions in acid solution. The same authors (67) also demonstrated an enzymatic demethylation of N,N-dimethyltyrosine-N-oxide in mouse liver homogenate leading to the formation of monomethyltyrosine and formaldehyde.

Demethylation of dimethylaminoazobenzene-N-oxide has been observed in the presence of iron alone (99), hemoglobin, or methemoglobin (196) and also with liver microsomes both in the presence (200) and in the absence (201, 202) of NADPH.

The substrate most extensively used in the study of enzymatic N-oxide dealkylation is N,N-dimethylaniline-N-oxide. This compound forms monomethylaniline and formaldehyde in the presence of liver microsomes (200, 201), possibly even in the absence of NADPH and oxygen (9). The reaction is decreased by cholate treatment of the microsomes (9) or by SKF 525-A (1).

Dimethylaniline-N-oxide was also used by Ziegler *et al.* (124, 125, 154, 211, 212), who have investigated N-oxide demethylation most extensively. This reaction was greatest in microsomes of liver, less in extrahepatic tissues, and still less in nonmammalian liver (124); it has been investigated primarily in microsomes of hog liver. The products of the reaction are equivalent amounts of formaldehyde and monomethylaniline (154), and the reaction velocity is proportional to incubation time and amount of microsomal protein. N-Oxide demethylation, according to the findings of these authors, depends on the presence of microsomes but not of NADPH, NADH, oxygen, or magnesium ions. The reaction is blocked by pretreatment of microsomes with heat or detergents; it is strongly inhibited by SKF 525-A, CO, & pyridine, but not by cyanide or azide; and it is much diminished by treatment of the microsomes with cholate or lipases, or by ageing them or freezing and thawing them. These procedures disrupt microsomal membranes or inactivate cytochrome P-450 by formation of cytochrome P-420. Participation of P-450 in N-oxide demethylation has therefore been proposed by the authors. This is particularly suggested by the inhibitory action of CO and SKF 525-A as well as by the increased rate of demethylation observed when microsomes of barbital-pretreated animals are used. Although heat-insensitive catalysis by hemoglobin and myoglobin has been observed, the microsomal reaction must be enzymatic because of its extreme heat lability. Whereas cytochrome P-450 is a typical mixed-function oxidase, the N-oxide demethylase is not likely to be an oxidase since the reaction is theoretically not an oxidation and does not require oxygen. An important finding concerns the substrate specificity of the microsomal N-oxide demethylase (125): incubation of microsomes with the N-oxides of dimethylaniline and four other compounds (N,N-dimethyl-1-naphthylamine-N-oxide, N,N-dimethyl-*p*-toluidine-N-oxide, *p*-chlorodimethylaniline-N-oxide, and N-ethyl-N-methylaniline-N-oxide) leads

to demethylation, while another four N-oxides (N,N-dimethylbenzylamine-N-oxide, N,N-dimethylcyclohexylamine-N-oxide, N,N-dimethyloctylamine-N-oxide, and morphine-N-oxide) are not demethylated. Susceptibility of these N-oxides to demethylation is not correlated with polarity or other obvious chemical characteristic.

Authors other than Ziegler *et al.* were also unable to show microsomal demethylation of certain N-oxides: only negligible demethylation occurs with the N-oxides of propoxyphene (130), chlorcyclizine (31, 111), chlorpromazine (44), and imipramine (19).

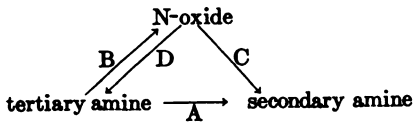
The N-oxide of imipramine (24, 26), however, is demethylated in liver homogenates without added cofactors, a system that does not demethylate the corresponding tertiary amine, imipramine. The same reaction has been observed in extrahepatic tissues; it is only partially heat-labile, is not dependent on oxygen, and can be carried out by hemoglobin or ferrous ions. The reaction is inhibited by oxygen but not by CO or SKF 525-A. Part of the liver activity can be localized in the nuclear fraction. These characteristics are identical with those of the reduction of this N-oxide (section VI A). Furthermore, a fairly constant demethylation/reduction ratio of approximately 0.1 is obtained under a wide variation of experimental conditions.

The results of the investigations of the few N-oxides studied so far do not allow definite conclusions with respect to N-oxide dealkylation. As in the case of N-oxide reduction, comparison of the reported studies shows discrepancies as to possible mechanisms, localization of the process, participation of heme catalysis, heat sensitivity, and action of inhibitors. It is not clear whether these discrepancies are due to methodological or species differences; however, it is likely that substrate differences play a role, *i.e.*, that not all N-oxides are dealkylated by a common mechanism. The problem of the relative polarity of N-oxides, which may possibly influence their availability for membrane-bound enzymes, has received little consideration. Finally, too little attention has been paid to a clear differentiation between direct N-oxide dealkylation on one hand and the sequence of N-oxide reduction and subsequent (direct) tertiary amine dealkylation on the other. In either case the net effect would be the formation of equivalent amounts of secondary amine and aldehyde. This problem is discussed further in the next section.

C. Position of N-oxides in drug metabolism

On the preceding pages several reactions occurring in biological systems have been described: formation of N-oxides from tertiary amines (reaction B), N-oxide reduction to tertiary amines (reaction D), demethylation of N-oxides to secondary amines (reaction C). Many substrates undergo more than one of these reactions simultaneously. Thus N-oxides must be considered not only as substrates or terminal metabolites but also as possible intermediate products. This may be particularly important in the case of oxidative N-dealkylation of tertiary amine drugs. This frequent and important microsomal drug metabolism reaction is not yet fully understood. A controversy has been carried on for many years as

to whether tertiary amines are demethylated directly by initial C-oxidation (reaction A), or by initial N-oxidation and subsequent N-oxide demethylation (reactions B and C). The four single reactions, A, B, C, and D, are represented in scheme I, which also shows the possible positions of N-oxides in the metabolism of amine drugs.



SCHEME I

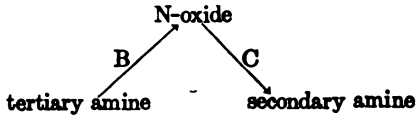
Several additional reactions could be added to this scheme. For example, the secondary amine can be further dealkylated, but this second dealkylation step is usually much weaker than the first (20, 128). Also negligible is N-methylation of secondary amines, observed in only a few cases (51). Depending on the nature of the radical R, extensive further metabolism of the tertiary or secondary amine may occur by microsomal hydroxylation or other pathways. The four reactions represented in scheme I are not meant to be single steps. There is evidence that reaction A can proceed through an intermediary N-methylol (hydroxymethyl) compound which is formed by aliphatic hydroxylation and is then eliminated as formaldehyde. Similarly reaction C could be understood as an initial rearrangement of the N-oxide to form the intermediary methylol compound. Since space does not allow the presentation of the many important findings and problems in the field of dealkylation, the reader is referred to the most informative review by McMahon (129).

The key problem concerning the situation depicted in scheme I is whether N-oxides are intermediates or merely by-products in oxidative N-dealkylation. Both views have been supported by several investigators. In the following paragraphs I will summarize and briefly comment on the pertinent findings supporting one or the other hypothesis.

Fish *et al.* (66, 67) first introduced the concept of oxidative N-dealkylation proceeding *via* intermediate N-oxides; they demonstrated the demethylation of N-oxides of N-dimethylated amino acids and biogenic amines in chemical model systems and in liver homogenate. However, their substrates were highly polar and in some instances naturally occurring compounds. It has been shown (178) that these compounds may be oxidized by mitochondrial monoamine oxidase, and experiments with $H_2{}^{18}O$ and labeled dimethyltryptamine demonstrated that N-oxides could be ruled out as intermediary metabolites of the oxidative deamination of tertiary amines. Therefore these substrates scarcely bear a significant resemblance to the lipophilic amine drugs in question.

The strongest support for the view of N-oxides as intermediates in N-demethylation stems from the extensive work of Ziegler *et al.* (49, 124, 125, 154, 212, 213). Their experiments involved incubation of N,N-dimethylaniline and its N-oxide with hog liver microsomal preparations. Under these conditions the rate of N-oxide demethylation is several times higher than that of overall demethylation of the tertiary amine. In addition, the rate of N-oxide formation is suffi-

ciently high for the N-oxide to be an intermediate in oxidative N-demethylation. Thus this process has been formulated as a B-C sequence as represented in scheme II.

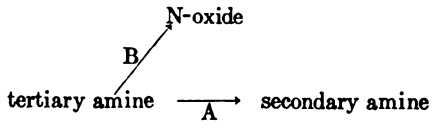


SCHEME II

Reactions B and C have been characterized by their different cofactor requirements and different responses to inhibitors. SKF 525-A inhibits C, but not B, and also increases N-oxide excretion of rats given tertiary amines. Finally the enzymes involved, tertiary amine N-oxidase and N-oxide demethylase, show the same distribution among organs and species.

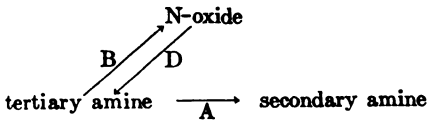
The interpretation of N-demethylation as a B-C sequence is, however, inferential rather than conclusive, since participation of the A reaction cannot be ruled out. In almost all experiments the formation of only N-oxide and formaldehyde, but of no other metabolites or intermediates, has been measured. Therefore the possibility of N-oxide reduction and hence N-oxide demethylation as a D-A sequence cannot be eliminated. For the same reason the characteristics ascribed to the B and C reactions could also hold for the B and A reactions. The extremely low apparent K_m value of dimethylaniline-N-oxide demethylase (1.39×10^{-1} M) may be interpreted as prohibitive for the demethylation of N-oxide formed in incubations of dimethylaniline. The increase by SKF 525-A of N-oxide excretion in rats treated with the tertiary amine may also be interpreted as a metabolic shift from the inhibited A reaction to the B reaction. In fact the significant decrease by SKF 525-A of N-oxide excretion in N-oxide-treated rats speaks against an involvement of the C reaction and in favor of a D-A sequence. Finally, the absence of C in the case of several N-oxides suggests the improbability of the B-C sequence as a general mechanism of N-dealkylation.

Additional indications against N-oxides as intermediates in N-demethylation have emerged from findings of other investigators. Gillette *et al.* (28, 75) found that demethylation of dimethylaniline-N-oxide proceeds at a slower rate than overall demethylation of the tertiary amine. He and other workers (26, 76) were also able to detect reduction of this N-oxide in liver preparations. Arrhenius (9) reported a marked excess substrate inhibition of overall demethylation of dimethylaniline but the absence of such an inhibition from both N-oxidation and N-oxide demethylation. Furthermore the demethylation rate of the tertiary amine is not influenced by the presence of a large pool of added N-oxide. These results favor alternative pathways of N-demethylation and N-oxidation, *i.e.*, demethylation would have to be considered as a process involving an initial carbon atom oxidation and N-oxides would be by-products, as indicated by scheme III. Studies carried out by other authors with chlorpromazine (44, 70, 84), propoxyphene (130), and their respective N-oxides point to the same conclusion.



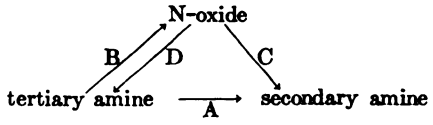
SCHEME III

An interesting study with respect to this problem has been performed by Burns, Kuntzman *et al.* (31, 111). These authors found that chlorcyclizine in rat liver 9000 × *g* supernatant is demethylated at a high rate whereas its N-oxide remains almost unchanged. *In vivo*, however, chlorcyclizine-N-oxide is transformed into both the tertiary and the secondary amine. Since their formation rates are influenced in a characteristic manner by SKF 525-A, the authors concluded that norchlorcyclizine must be formed from the N-oxide by a D-A sequence. Since chlorcyclizine is also N-oxidized, the situation must be assumed to be as depicted in scheme IV. Studies with hydroxyzine (43) point in the same direction.



SCHEME IV

Bickel *et al.* (19, 21, 24, 26) have investigated the metabolism of imipramine and its N-oxide. Rat liver 9000 × *g* supernatant, with added cofactors, in amounts that rapidly N-demethylate and N-oxidize imipramine, does not metabolize imipramine-N-oxide. On the other hand homogenates of liver or extrahepatic tissues without added cofactors reduce and demethylate the N-oxide, but do not metabolize the tertiary or secondary amine. As expected, all four reactions, A, B, C, and D, proceed in liver homogenate plus added cofactors, *i.e.*, the combination of the above systems. This has been proved by the use of imipramine as labeled substrate and its N-oxide as unlabeled pool and *vice versa*. This situation is summarized in scheme V.



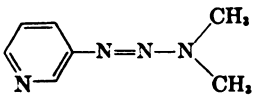
SCHEME V

The experiments with labeled substrates also showed that the rates of the B and C reactions are relatively slow whereas the D reaction proceeds very rapidly. Thus N-demethylation is predominantly an A reaction, since most of the N-oxide formed is reduced back to the tertiary amine. These results, which lead to the formulation of scheme V, are also consistent with the observations leading to the schemes III and IV. However, the question of whether the metabolic fate of N-oxide added exogenously is identical with that of N-oxide produced inside the lipoid barrier of the microsomal membrane has not yet been answered and might influence further work.

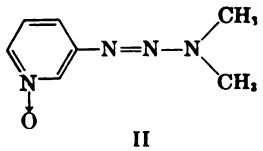
On the basis of data available today it seems likely that N-oxidation and N-dealkylation are alternative pathways, *i.e.*, that N-oxides are by-products rather than intermediary products. The dealkylation of compounds unable to form N-oxides lends further support to this view. Compounds of this type are N-methyl barbiturates (32), secondary amines, and O-methylated compounds. Dealkylation is thus best understood as an initial microsomal C-hydroxylation, which results in the formation of a methylol intermediate; the methylol group would then be expelled as formaldehyde. This view explains the close relationship between dealkylation and microsomal hydroxylations without introducing problems in the case of secondary amines, amides, or O-alkylated compounds. The assumption of alternative pathways for N-dealkylation and N-oxidation also explains the findings that indicate that the latter reaction, unlike dealkylation, does not depend on the microsomal electron transport chain with NADPH-cytochrome c reductase and cytochrome P-450.

Much work is still needed to clarify this and related problems. Comparative studies with many substrates will be necessary to prove the existence of either a common pathway for N-dealkylation or characteristic substrate specificities. The multitude of valuable data now available will yield better clues only when reproduced under comparable conditions with respect to animal species and methodology. Better differentiation and enzymatic characterization of the single reactions is obviously needed. Although N-dealkylation of a large number of substrates has been well studied, far too little is known about N-oxidation and even less about reduction and dealkylation of N-oxides.

A serious lack of knowledge exists as to whether N-oxides are available to membrane-bound microsomal enzymes: if a lipophilic tertiary amine is transformed into its N-oxide within the membrane, the translocation and metabolic fate of this N-oxide may be different from the fate of the same N-oxide when added exogeneously. It is possible that the access to microsomal membrane-bound enzymes could be estimated by the study of binding to microsomes; *e.g.*, propoxyphene-N-oxide shows a certain degree of binding and has been denoted as "appreciably lipid soluble" (130) since, like imipramine-N-oxide (23), it is readily extracted in chloroform. However, no information seems to be available about relative polarities of N-oxides. Specially designed model compounds may be helpful in this respect. A recently published example (169) is 1-(pyridyl-3)-3,3-dimethyl-triazene (I) and its N-oxide (II). Both compounds possess the same dimethylamine group susceptible to demethylation, whereas the N-oxidized nitrogen is at a different site. Compound I but not II is demethylated. Thus a separation between metabolically vulnerable groups and groups determining only polarity and translocation may be achieved. No final answer concerning the possible role of N-oxides as intermediary metabolites can be given until their access to specialized enzymes has been elucidated. This holds true despite agree-



I



II

ment among most authors that N-oxides are minor metabolites both *in vitro* and *in vivo*, since recent findings (15, 26) have shown that the use of adequate methods and suitable conditions *in vitro* can lead to amounts of N-oxides surpassing those of other metabolites such as demethylated or hydroxylated products. If one considers N-oxide reduction, the above finding would be compatible with the low amounts of N-oxides usually found as terminal excretion products. Thus a cyclic interconversion between tertiary amine and N-oxide may occur as indicated in scheme VI. Such a situation, *i.e.*, the transitory formation of considerable amounts of N-oxide, could have pharmacological if not toxicological implications.

SCHEME VI

VII. SUMMARY AND CONCLUDING REMARKS

N-Oxides are oxidation products of tertiary amines. They were detected as a new class of chemical compounds before 1900 and were later found to occur as constituents of living matter. In recent years an increasing number of studies by biomedical scientists has been devoted to N-oxides for several reasons. The natural occurrence of N-oxides in plant and animal tissues has posed interesting problems as to the biochemistry and function of these compounds in biological systems. In several cases N-oxides are more active than their corresponding tertiary amines. Hence several N-oxides are important as pharmacological or toxicological agents, *e.g.*, in the groups of alkaloids, chemotherapeutics, antibiotics, psychotropic drugs, methemoglobin forming compounds, and carcinogenic agents. Certain oncogenic N-oxides act as antimetabolites and have been postulated as inducers of spontaneous cancer. Finally, a large number of tertiary amine drugs produce N-oxides as metabolites or intermediates in drug metabolism.

N-Oxides have been detected in animals, plants, and microorganisms. Trimethylamine-N-oxide, which occurs primarily in marine species but also in mammals, including man, has received most attention; however, its biosynthesis and its physiological functions have not yet been fully elucidated. In addition to the pharmacologically active N-oxides of natural origin, many others have been prepared synthetically.

Many findings suggest that N-oxides play an important role in the metabolism of drugs. In addition to N-oxidation of tertiary amines, N-dealkylation of N-oxides has been detected. This suggested a pathway of oxidative N-dealkylation of tertiary amines in which N-oxides would be intermediate metabolites. On the other hand strong evidence for a direct tertiary amine N-dealkylation has also been presented; in this case N-oxides would be by-products. However, N-oxides could also be intermediate metabolites because of the frequently occurring N-oxide reduction which transforms them into the corresponding tertiary

amines. With some substrates several or all of these four reactions have been observed to occur simultaneously. Despite many investigations carried out in an attempt to elucidate the mechanisms and enzymatic characteristics of these reactions, our knowledge is still incomplete.

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