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Metabolic *N*-oxidation of secondary and primary aromatic amines as a route to ring hydroxylation, to various *N*-oxygenated products, and to dealkylation of secondary amines

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We now propose a general metabolic *N*-oxidation complex leading to the nitroso compound and to *p*- and *o*-ring hydroxy derivatives of aromatic amines and also by further metabolism to the hydroxylamine from the amine. The involvement of a similar complex and scheme explains the metabolism of *N*-alkyl-aromatic amines to yield secondary and primary hydroxylamines, nitrones and ring hydroxy amines.

The schemes are analogous to those proposed by us [1] for the metabolism of primary and secondary aliphatic amines but with some differences because of the involvement of the aromatic ring for aromatic amines.

It is probable that the same enzymes are involved in the oxidation of both the aromatic and aliphatic primary and secondary amines; the evidence is as follows:

(a) Aromatic primary and secondary amines of various types e.g. *p*-chloroaniline, phenothiazine, 2-chlorophenothiazine, 1-naphthylamine and iminodibenzyl inhibited the microsomal *N*-oxidation of phentermine (Beckett *et al.* unpublished) and the latter inhibited the microsomal oxidation of a range of diverse aliphatic primary and secondary aliphatic amines [1].

(b) The secondary aromatic amines, *N*-methyl- and *N*-ethyl-aniline are metabolised by microsomes to the primary hydroxylamine, phenyl-hydroxylamine, faster than is their parent primary amine, aniline [2]; similarly the secondary aliphatic amine, mephentermine, is metabolised faster to the primary hydroxylamine than is the primary aliphatic amine, phentermine [1].

(c) The inhibitor of oxidation of C located α to basic N atoms or of ring oxidation i.e. SKF 525A did not inhibit the microsomal formation of nitrosobenzene from *N*-alkylanilines [3], nor did it inhibit greatly the *N*-oxidation of the aliphatic amines, phentermine or mephentermine [1].

The proposed scheme for the *N*-oxidation for primary amines and the subsequent reactions is shown in Fig. 1. One of the electrons from the N lone pair is transferred to convert oxygen in the triplet state to the singlet state via the mediation of a flavoprotein; the complex of the nitrogen radical cation with the flavoprotein/oxygen (II) is formed. Two routes for change of II are available. One involves reduction of the complex to form the complex III containing the anion radical with the reduced flavoprotein. This complex (III) then produces the zwitterion-reduced flavoprotein complex (IV); reduction within the complex of the zwitterion directly to VI or after proton rearrangement to give the *N*-hydroperoxide complex (V), yields the primary hydroxylamine (VII) with regeneration of the flavoprotein and elimination of water. The oxygen atom of the primary hydroxylamine (VII) is then derived from atmospheric oxygen.

The other route from II involves dissociation of the complex to give flavoprotein and the zwitterion (VIII) which upon proton rearrangement yields the *N*-hydroperoxide (IX) which can change chemically in neutral aqueous solution by three different routes. By route a, electron attraction towards the oxygen atoms of the *N*-hydroperoxide facilitates attack from the OH^- ion from water (or X^- ion from negative ions in solution) in the *p*-position of the ring to yield the quinoid-type structure (X) which upon proton rearrangement yields the *p*-hydroxy primary amine (XI). Similarly route b yields the *o*-hydroxy amine (XIII) by a similar mechanism. Route c will yield the nitroso compound (XIV) and the oxygen atom of this compound is derived from air. Obviously substitution on the ring will alter the importance of routes a and b to each other and to route c; electron attracting groups on the ring will be expected to facilitate a or b at the expense of c. The oxygen atom of the ring OH groups is derived from the water.

Subsequent to these changes, the metabolic products XI, XIII, XIV and VII may be further metabolised e.g. the nitroso compound (XIV) can be reduced metabolically to VII which can be both oxidised further by oxidation of the OH group and rearrangement to yield *p*- and *o*-hydroxy-*N*-hydroxy compounds or by reduction back to the parent amine (1).

It is postulated that, in a similar manner, (see Fig. 2) a secondary aromatic amine (XV) is oxidised to the free radical ion complex (XVI) which can be reduced to other complexes XVII, XVIII and XIX, which then yield the secondary hydroxylamine (XXI), the oxygen in this compound being derived from the air. Also dissociation of the complex (XVI) gives the zwitterion (XXII) which upon proton rearrangement yields the unstable *N*-hydroperoxide (XXIII). This hydroperoxide then is attacked by OH^- ions (or other X^- ions from solution) to yield *p*- and *o*-ring hydroxy secondary amines XXV and XXVII as indicated, the oxygen atoms of the hydroxy groups thus being derived from water.

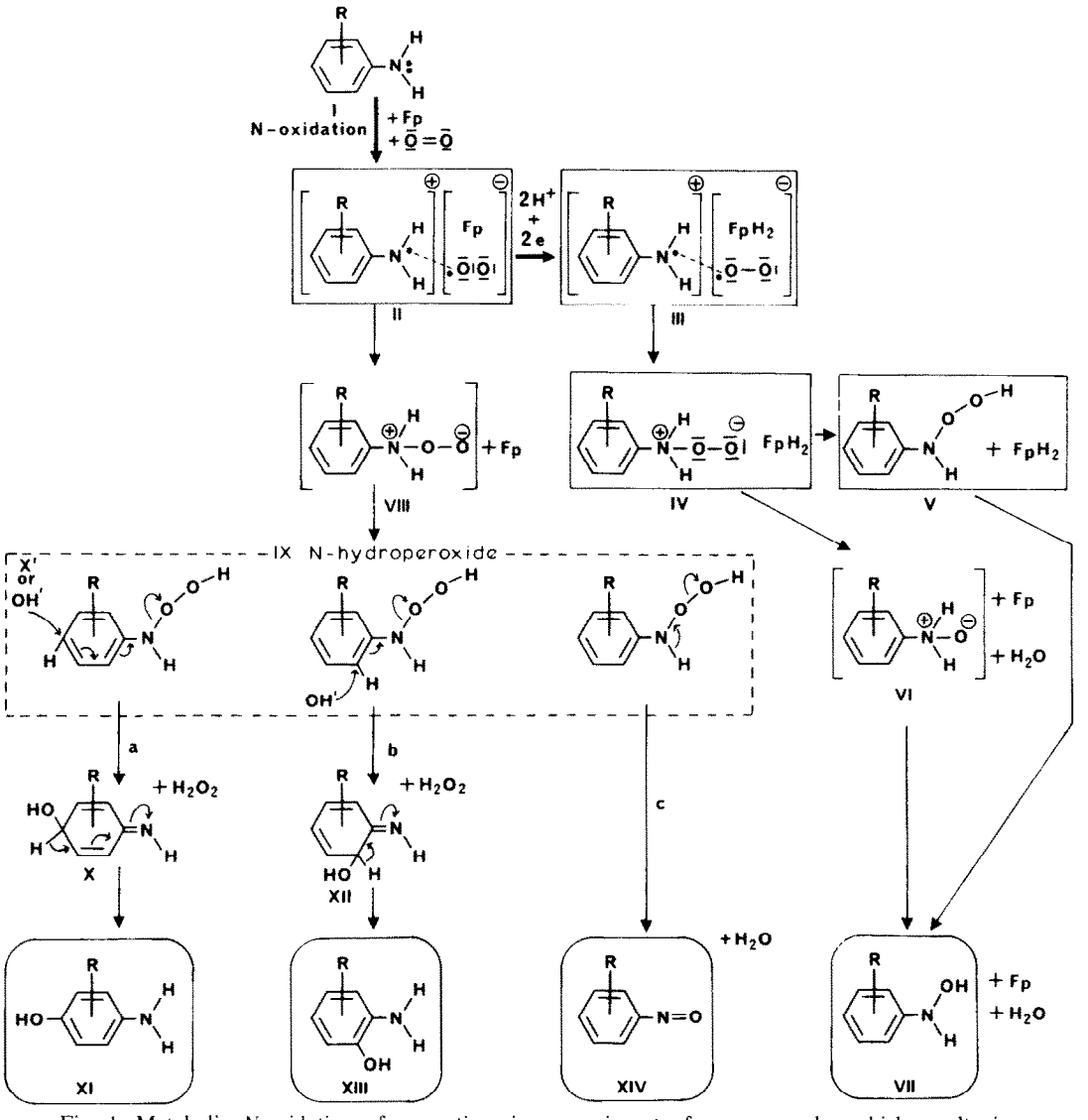

Fig. 1. Metabolic *N*-oxidation of aromatic primary amines to form a complex which results in subsequent changes under neutral aqueous conditions to yield ring hydroxy compounds (O from H_2O) or nitroso compounds (O from air) and also, by metabolic reduction, the primary hydroxylamines (O from air).

□ = complexes, [ ] = unstable, ⌒ = compounds isolated → = metabolic routes, ⇢ = chemical changes

By route c, the *N*-hydroperoxide (XXIII) is converted to the nitrone (XXVIII), the oxygen atom of which is thus derived from the air*. If the *N*-alkyl group is small, i.e. R′ in XV equal to H, then the nitrone (XXVIII) will be slowly decomposed in water to give unstable XXIX which then immediately yields the primary hydroxylamine (XXX) (O from air) with elimination of the original *N*-alkyl group-CH_2R' as the aldehyde, R′CHO, the oxygen of which is derived from the water.

The following experimental results are in accord with the above schemes.

1. *N*-hydroperoxides have been isolated after the metabolism of the secondary aromatic amines, phenothiazines and 2-chlorophenothiazines [4].

2. Incubation of a series of *N*-alkylanilines i.e. secondary aromatic amines, with liver hepatic fractions yielded *p*-hydroxy compounds whereas their corresponding tertiary amines did not under similar conditions [5]. Thus *p*-hydroxylation of secondary aromatic amines is a consequence of *N*-oxidation rather than metabolic ring hydroxylation.

3. The ratio of *p*-hydroxyaniline to phenylhydroxylamine produced by metabolism of aniline and *N*-ethylaniline was independent of the microsomal content of the medium. This ratio was the same at 10 and 40 min of incubation and was not altered by changing the concentration of the NADPH generating system used [6]. Thus a single chemical entity is indicated as the precursor of the *p*-hydroxy amine and the primary hydroxylamine.

4. Secondary aromatic amines, i.e. methyl and ethyl aniline are oxidised metabolically to the primary hydroxylamine, phenylhydroxylamine at faster rates than is the par-

* This nitrone may be formed from the *N*-hydroperoxide by the ionic mechanism indicated in Fig. 2 or by homolytic fission of the O—O bond and the α—C—H bond to yield a molecule of water and a biradical which undergoes electron redistribution (see Fig. 3).

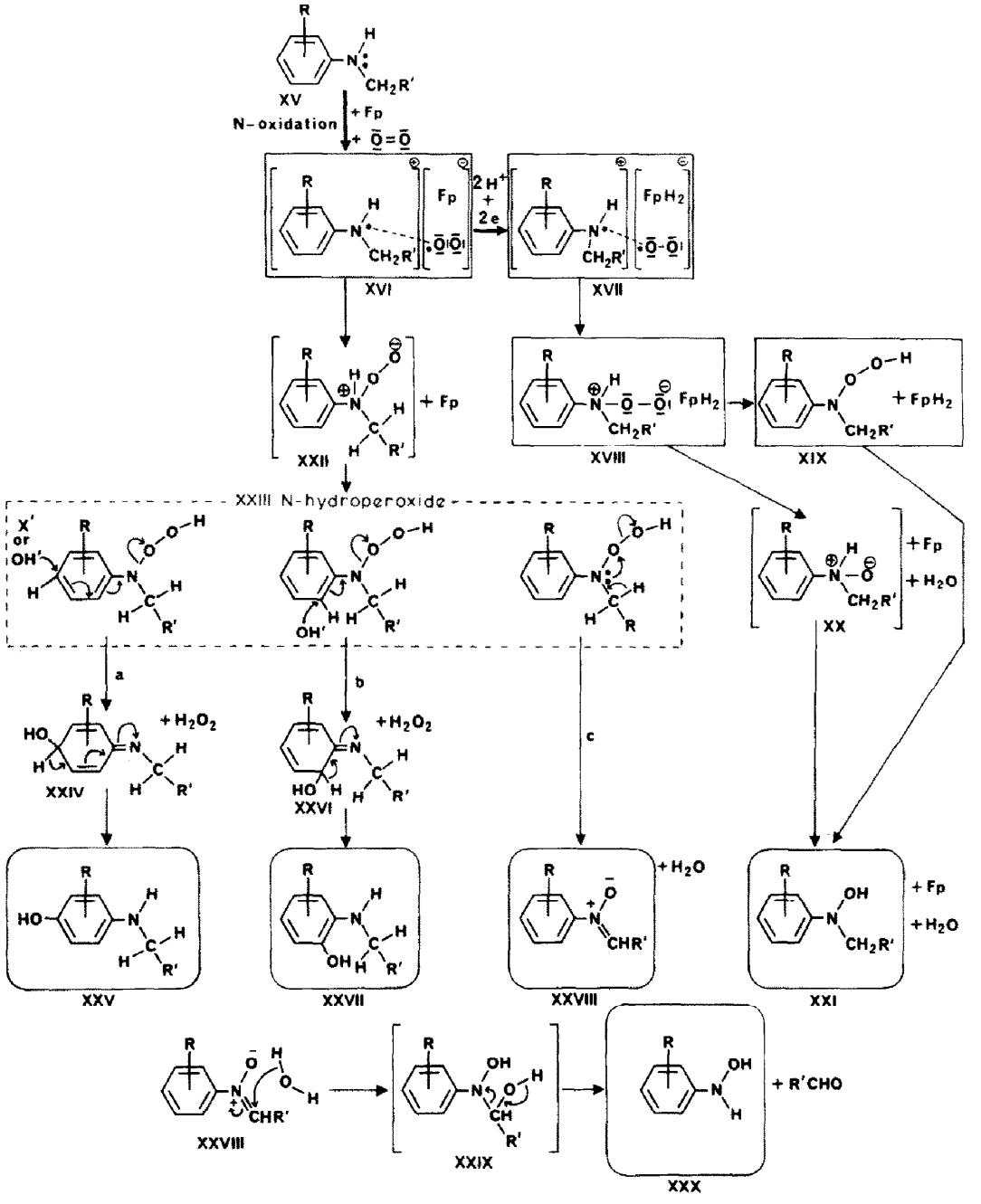

Fig. 2. Metabolic *N*-oxidation of aromatic secondary amines to form a complex which results in subsequent changes under neutral aqueous conditions to yield ring hydroxy compounds (O from H_2O) or nitrones (O from air) and primary hydroxylamines (O from air) and also, by metabolic reduction, the secondary hydroxylamines (O from air).

□ = complexes, [ ] = unstable, ◯ = compounds isolated → = metabolic routes, ⟶ = chemical changes

cnt primary amine, aniline [2]. Thus the primary hydroxylamine produced metabolically from methyl and ethyl aniline is not produced primarily via metabolic oxidation of the primary amine produced by *N*-dealkylation.

Increase in the length of the *N*-alkyl group decreases the rate of phenylhydroxylamine formation but increases the rate of *p*-hydroxylation [2]. This result is explicable in terms of an increase in stability of the nitrones (XXVIII) as the *N*-alkyl group is increased with consequent progressively less breakdown to the primary hydroxylamine (XXX).

5. The introduction of substituents in the ring which decrease the amount of aromatic hydroxy compounds produced upon incubation, cause an increase in the amount of *N*-hydroxy compounds produced [3]. This can be explained by the common *N*-hydroperoxide intermediate

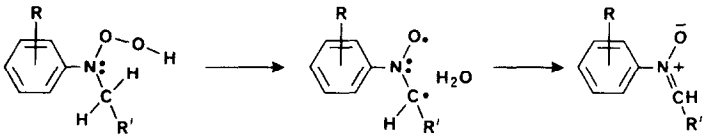

Fig. 3. Free radical mechanism of formation of nitrones from *N*-hydroperoxide intermediates.

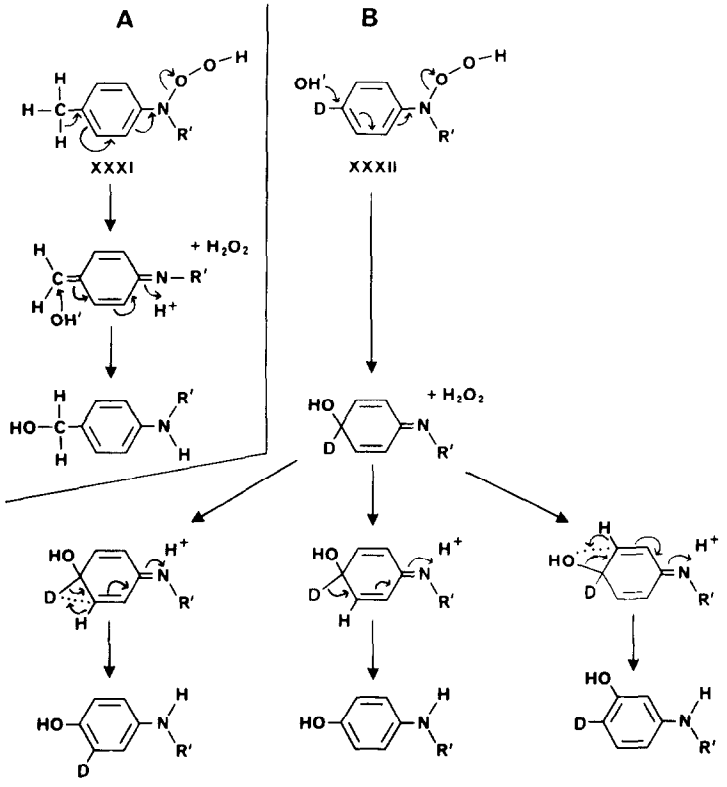

Fig. 4. Implications of the *N*-hydroperoxide metabolic route in (A) the oxidation of a *p*-methyl substituent and (B) the migration of a *p*-substituent (NIH shift) in secondary and primary (R′ = H) aromatic amines.

which may be influenced towards one or the other route of chemical change (see Figs 1 and 2) by the nature of the added ring substituent.

6. Aromatic amines are oxidised metabolically to their corresponding nitroso compounds [3].

7. Aniline is oxidised metabolically to *p*-hydroxyaniline under conditions in which phenylhydroxylamine does not rearrange to *p*-hydroxyaniline [7].

8. Incubation of *N*-alkylanilines with liver microsomes gave nitrosobenzene as well as dealkylation to aniline [3].

The above schemes have implications for the metabolic replacement of *p*-substituents in aromatic amines and for the NIH shift in these compounds. It would be expected that *p*-methyl group would be oxidised to a hydroxylmethyl group by chemical attack upon the *N*-hydroperoxide compound (XXXI) as shown in Fig. 3a rather than undergo replacement of the methyl group by the hydroxyl group or methyl migration to the *m*-position. Although it is reported [3] that the oxygen atom introduced into the aromatic ring in metabolic reactions involving the NIH shift is from the air, the above schemes indicate the possible role for the *N*-hydroperoxide as in Fig. 3b in the NIH shift in the metabolism of primary and secondary amines but involving the oxygen atom from water.

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