Table 3. Solute content of granules dried in a fixed bed.

Mean depth below bed surface (mm)	Salt content mg per g dry solid (1)	(2)	(3)	(4)	(5)	(6)
1·65	1·43	14·2	1·50	15·4	25·1	32·3
4·95	1·45	14·7	1·48	15·1	26·5	27·8
8·25	1·47	15·7	1·47	14·7	30·8	25·5
11·55	1·49	15·5	1·45	14·5	34·0	22·8
14·85	1·40	14·4	1·43	14·3	16·5	22·9
18·15	1·39	13·1	1·42	14·2	7·4	22·2

(1), (2) Beds dried by infrared radiation at peak wavelength 2·8 μm for 60 min.
(3), (4) Beds dried by convection with warm air at 37 °C for 7 days.
(5) Kaolin-based granule dried by infrared as beds (1) and (2) (Travers 1975).
(6) Ditto dried by convection in air at 37 °C for 2 days (Travers 1975).

Each figure is the mean of two determinations under the stated conditions.

We therefore consider that migration effects similar to those operating in the kaolin-based system also account for the solute distribution in these lactose granulates.

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Disappearing *N*-hydroxy compounds

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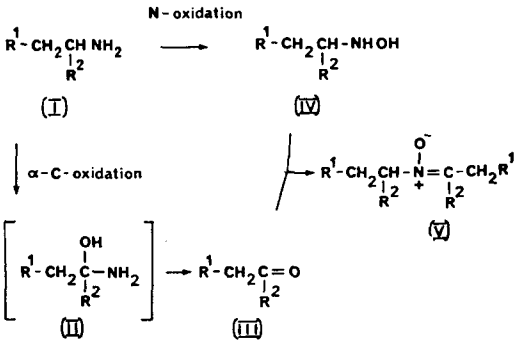
Although metabolic *N*-oxidation of both primary and secondary aliphatic and aromatic amines to give hydroxylamines has been well established (see Weisburger & Weisburger 1973; Coutts & Beckett 1977, for reviews), the presence of these relatively unstable compounds in biological fluids has been questioned. Even when their presence has been demonstrated, it is possible that the amounts present have been greatly underestimated for the following reasons:

Condensation following metabolism. Metabolic oxidation of aliphatic *N*-centres e.g. (Scheme 1) in drugs and endogenous amines involves α-*C*-oxidation yielding ketones (III: R^2 = alkyl) and aldehydes (III: R^2 = H), via alkanolamines (II), as well as *N*-oxidation to give hydroxylamines (IV). Thus compounds (i.e. carbonyl compounds and hydroxylamines) with potential for mutual condensation to produce nitrones (V) are formed by the same general oxidative process in the same biological tissues (Scheme 1).

There is some evidence that either the same active sites or closely associated active sites are involved in both oxidation routes (Beckett 1978). Also since *N*-oxidation and deamination occur in brain tissue (as well as in liver and kidney) such nitrones may be produced locally in the c.n.s. as well as at many other sites.

Under simulated biological conditions and concentrations i.e. in either aqueous phase, e.g. phosphate buffer pH 7·4, or lipid medium e.g. chloroform, we demonstrated that *N*-hydroxydidesmethyl imipramine, -chlorimipramine, -promazine and -chlorpromazine, readily combined with phenylacetaldehyde (the product of deamination of phenethylamine an endogenous amine) and also acetaldehyde, from the metabolic oxidation of ethanol, to give the corresponding nitrones. Also, metabolically-produced primary hydroxylamines of mexiletene, norfenfluramine, amphetamine and phenethylamine condensed with their products of deamination to give the corresponding nitrones. These products were identified by comparison with authentic materials (t.l.c. and m.s. evidence).

Condensation reactions of amines, drugs or endogenous compounds, with aldehydes (derived from various sources) have previously been reported (see Jenner & Testa 1978). For example, dopamine condenses with its deaminated product and with acetaldehyde to give tetrahydropapaveroline and the related alkaloid salsolinol respectively (Scheme 2).



Scheme 1. The metabolic formation of N-oxidation and deamination products with potential for mutual condensation to give nitrones. R^1 = aryl, aralkyl or alkyl; R^2 = alkyl or H; [ ] indicates unstable compounds.

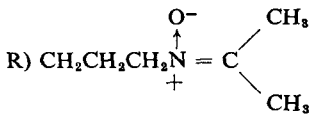
* Correspondence

These compounds have been demonstrated in the urine of patients receiving L-dopa (Sandler et al 1973; Cashaw et al 1974), and are known to possess pharmacological activity (Davis et al 1970). A pharmacologically active cyclic compound, the product of condensation of adrenaline with acetaldehyde, has also been demonstrated in vitro (Osswald et al 1975); furthermore, this compound has been shown to have hepatotoxic capacity in the rat (Moura et al 1977).

Condensation during isolation. Before analysis metabolic products are usually extracted into an organic phase such as ether. It has been shown that even after careful distillation this contains aldehydic impurities, e.g. formaldehyde, acetaldehyde, propionaldehyde (Beckett et al 1978). Although bisulphite may be used to remove aldehydic impurities and the ether then distilled, the distillate rapidly develops peroxides and aldehydes.

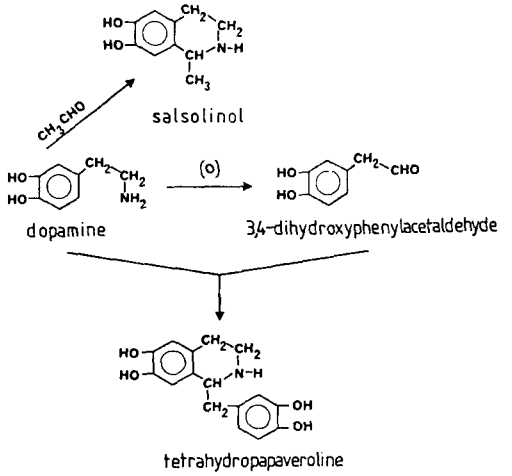
We have found, on examination by t.l.c. (solvent system $CHCl_3$(9):MeOH(1)), that after extraction of the above hydroxylamines with (A) freshly distilled Analar ether, (B) freshly distilled bisulphite-treated ether and (C) as (B) but containing 30 μg per ml of acetaldehyde, only minor amounts of hydroxylamines could be detected and extensive decomposition was observed. Methanolic solutions of the same hydroxylamines did not show decomposition when analysed under similar conditions.

Acetone is frequently used as one of the components of the solvent systems used in t.l.c. analyses (Moffat 1975). The presence of even small amounts of this solvent (or related solvents) converts the primary hydroxylamines completely to their corresponding 'acetone-nitrones' as indicated by t.l.c. and m.s. evidence (Beckett & Navas 1978).



These considerations may explain why hydroxylamines can be readily demonstrated in microsomal experiments under the appropriate conditions (Beckett et al 1974; Beckett & Haya 1977), but are not readily demonstrated in vivo unless converted to a conjugate. Also as nitrones are bound covalently to proteins, the present information raises serious questions about the basis of toxicity of certain amines.

The results of this study cast doubt on much of the published work on the metabolism of aliphatic amines and especially of primary aliphatic amines whenever *N*-oxidaton or deamination or rate of turnover of these compounds is considered, i.e. quantitative measurement of primary metabolites such as (III) or (IV) will give values which are lower than if nitrone formation did *not* occur. Also, whenever the *N*-oxidation and α-*C*-oxidation routes are considered in different species, quantitative aspects are suspect if nitrones are not measured since the 'apparent' quantity of α-*C*-oxidation (or *N*-oxidation) products may be overlooked if the species is a good *N*-oxidizer and a poor α-*C*-oxidizer (or vice versa) because the products in lower molar ratios will be converted completely to nitrones.



Scheme 2. Condensation of dopamine with acetaldehyde and its deaminated product to give cyclic alkaloids.

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