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Biodegradation of indoles

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Abstract

Indole and its derivatives form a class of toxic recalcitrant environmental pollutants The pathways for the biodegradation of indoles in microorganisms, plants and animals are discussed in detail. Various hydroxylation and detoxification reactions of indoles in these organisms are presented. Studies carried out on toxicity and mutagenicity of indoles are briefly discussed.

Key words: Indoles, N-aromatic compounds, pollutants.

1. Introduction

Indole and its derivatives form an important class of polycyclic N-aromatic compounds which are ubiquitous in nature. They participate in a variety of biochemical functions, as plant hormones, neurotransmitters, in addition to their role as building blocks in proteins and at active site of enzymes. Indole ring is also present in many important alkaloids, such as harmaline, reserpine, ajmaline, etc. A number of genetic disorders involving the enzymes of indole metabolism have been reported in man[1–3].

Indole and its derivatives are toxic and mutagenic in nature[4–11] and are now being considered as pollutants, as they are released into the environment through smoke, industrial effluents and sewage[12–18]. In order to maintain the ecological balance, several microorganisms degrade these compounds. Molecular oxygen, in addition to its role as the terminal electron acceptor, plays a pivotal role in the mineralization of these compounds. The enzymes which act on these aromatic compounds, activate the molecular oxygen and hydroxylate the hydrophobic substances[19]. A series of oxygenation reactions lead to the conversion of the aromatic compounds into simple metabolites which usually enter the tricarboxylic acid cycle. These reactions therefore culminate in the complete mineralization of the toxic compounds[20]. Plants and animals most often interconvert indole derivatives for metabolic purpose. In this review degradation of indoles by microorganisms, plants and

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animals is discussed. The literature on the uses of indole, its toxicity, mutagenicity and effect on other biochemical functions is also briefly highlighted.

2. Biodegradation of indole

Hydroxylation is a cardinal step in the mineralization of aromatic compounds. It helps in the solubilization of the hydrophobic aromatics and makes them accessible for further enzymatic reactions[19]. Microorganisms metabolize polycyclic N-aromatics by hydroxylating them at various positions. Bacteria are capable of utilizing them as carbon sources and hence degrade them completely. Fungi, in general, are not known to use them as a major energy source, but as a cometabolite. Some of these hydroxylated compounds are conjugated with glucuronide or sulfate prior to excretion. Animals detoxify xenobiotics by the cytochrome P-450 system-dependent hydroxylation which leads to the production of less toxic compounds or in some cases might give 'active products' which are carcinogenic[21].

2.1. *Degradation by microorganisms*

Indole is produced by several bacteria during the degradation of tryptophan. The formation of indole from tryptophan by the action of tryptophanase is an important taxonomic criterion for the identification of bacterial families, such as, *Enterobacteriaceae* and *Closteridium*. However, these bacteria apparently are only able to metabolize the side chain of the tryptophan molecule, leaving behind the heterocyclic ring as an excretory product. Indole thus formed is degraded by other microbes. Attempts to determine the metabolic fate of indole in microorganisms were initiated as early as 1920s. Raistrick and Clark[22] were the first to report the decomposition of indole by *Bacillus fluorescens* and some pseudomonads. Later, Supnieswki[23] isolated some strains of pseudomonads capable of degrading indole. An efficient bacterium for utilizing indole was also isolated from air[24]. However, it was only after 1950 that a reasonably satisfactory understanding of indole metabolism has emerged.

Sakamoto *et al*[25] isolated a gram-negative bacterium from tapwater which was capable of growing on indole as the sole source of carbon. The putative intermediates in the pathway for the indole degradation were used as substrates for growing the bacteria which was isolated by the simultaneous adaptation method of Stanier[26]. The oxygen uptake studies revealed these compounds as true intermediates in the pathway. The detection of indigotin in the spent medium suggested that 3-hydroxyindole (indoxyl) could be a precursor for indigo. These workers postulated that the degradation of indole proceeds *via* the initial hydroxylation to indoxyl, followed by a second hydroxylation to 2,3-dihydroxyindole and subsequent formation of isatin, N-formylanthranilate, anthranilate, salicylate and catechol (Fig. 1). The end product of the aromatic pathway, catechol, was cleaved by a dioxygenase to ultimately yield intermediates which could be metabolized through the tricarboxylic acid cycle. They ruled out the possibility of 3- and 5-hydroxyanthranilate and nicotinic acid being the intermediates in the metabolic pathway of indole[25]. These studies only involved the measurement of oxygen uptake in the presence of possible intermediates and hence the identification of intermediates was not unequivocal.

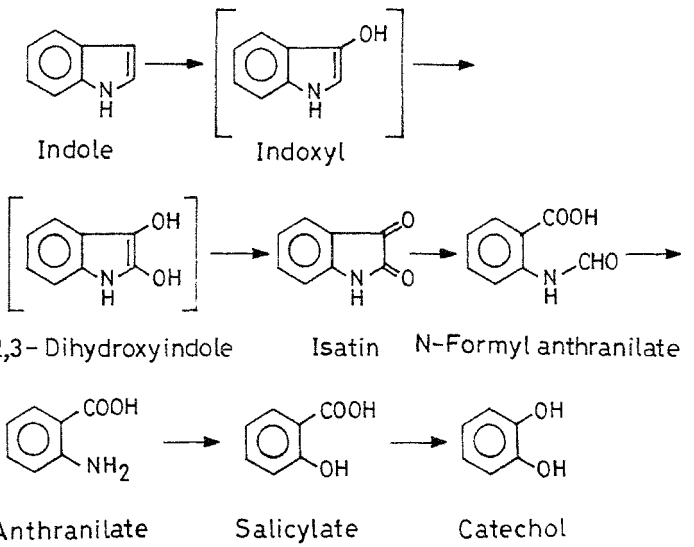

FIG. 1. Proposed pathway for the degradation of indole by a tapwater bacterium.

Fujioka and Wada[27] isolated a gram-positive coccus from soil which used indole as the sole source of carbon and nitrogen. 2,3-Dihydroxyindole was proposed as the first intermediate in the pathway. Direct evidence for the formation of dihydroxyindole was not obtained since indole was oxidized to anthranilate without the accumulation of this compound in the medium. However, when the cells adapted to indole were incubated with skatole (3-methylindole), the compound was oxidized with the consumption of one mol of oxygen per mol of substrate to form (+)-2-oxo-3-methyl, 3-hydroxyindole. This was not further metabolized by the organism and accumulated in the medium. They were unable to demonstrate the enzyme catalyzing the double hydroxylation. However, the dihydroxyindole oxygenase was partially purified and inhibition studies indicated the presence of Fe^{2+}. This enzyme was specific for dihydroxindole. Other related compounds such as indole, isatin, oxindole (2-hydroxyindole), 2-oxo-3-methyl, 3-hydroxyindole and catechol were unable to function as substrates. The authors postulated an epoxide mechanism, rather than a cyclic peroxide as an intermediate to explain the formation of 2-oxo-3-methyl-3-hydroxyindole from skatole[27].

The dihydroxyindole was oxidatively metabolized by direct ring cleavage by a dioxygenation reaction to yield an unstable N-carboxyanthranilate which was later converted to

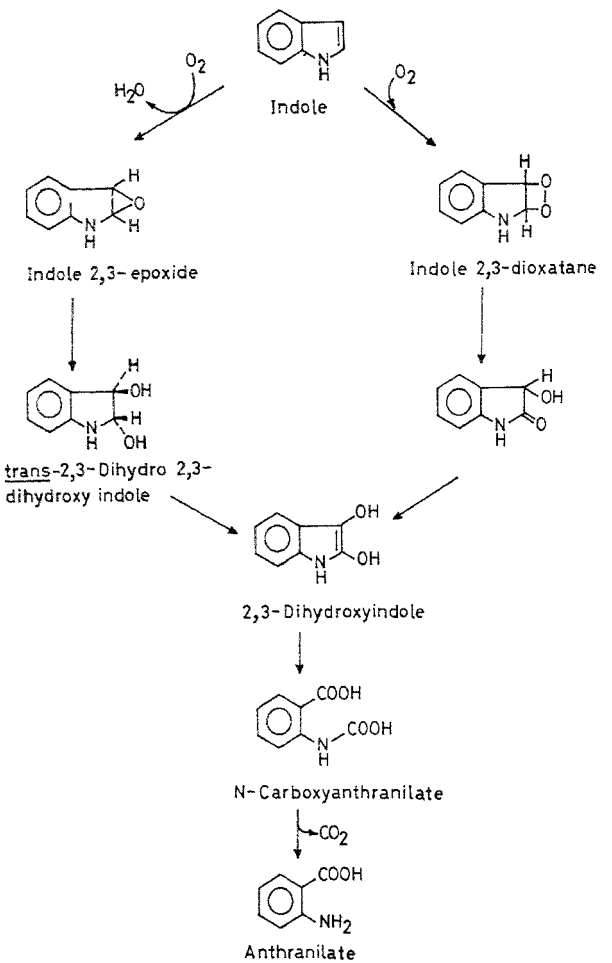

FIG. 2. Proposed pathway for the degradation of indole by a coccus.

anthranilate. Unlike the tapwater bacterium[25], Fujioka and Wada[27] were unable to show oxygen uptake with isatin or N-carboxyanthranilate indicating that the conversion of hydroxyindole to anthranilate was probably occurring in a single enzymatic step or in a concerted manner (Fig. 2).

In 1983, Claus and Kutzner[28] reported the isolation from the activated sludge of an *Alcaligenes* species of bacterium which utilized indole as the sole source of carbon. Two of the metabolites, anthranilate and indigo, were isolated from the spent medium but they failed to detect isatin and gentisate (2,5-dihydroxybenzoate). The oxygen uptake studies with the whole cells and detection of gentisate dioxygenase in the cell-free extracts enabled the authors to propose a pathway for degradation of indole *via* indoxyl → isatin → anthranilate → gentisate. *Alcaligenes* could also grow on indole 3-aldehyde and indoleacetic acid without the formation of indole and hence it was postulated that the degradation of these compounds would bypass indole and indoxyl. However, for all the three indolic compounds gentisate was the substrate for the ring cleavage. The pathways for degradation of indole and its derivatives are shown in Fig. 3[28].

To date there are only three reports on the aerobic degradation of indole in bacteria. It is interesting to note that each of them metabolized indole by different pathways, highlighting the diverse mechanisms employed in handling a single compound.

Recently, attention has been focussed on the degradation of indole by anaerobic and denitrifying bacteria. Wang *et al*[29] showed that indole could be anaerobically degraded by a consortium of methanogenic bacteria. Over a period of seven to eighteen weeks, this bacteria utilized the indole provided in the medium. A stoichiometry of

$$C_8H_5N + 8H_2O \rightarrow 3.5\,CO_2 + 4{\cdot}5\,CH_4 + NH_3 + OH^-$$

was shown for methane formation. Even though the rate of degradation was slow, the efficiency was about 84%. Berry *et al*[30,31] showed that indole was converted to oxindole under methanogenic conditions. The various conditions required for such reactions have been worked out[32]. Recently, a new pathway *via* oxindole, isatin and anthranilate was proposed for indole metabolism in denitrifying microbial community[33].

Many fungi are known to be as efficient as bacteria in degrading recalcitrant xenobiotics. Studies on the degradation of indole by a versatile soil fungus, *Aspergillus niger*, carried out in this laboratory showed that indole significantly inhibited the growth of *A. niger*[34,35]. The fungus showed a lag phase lasting for about 30 hours, even when there was 500-fold excess glucose present in the medium compared with 0.02% indole. Indole was cometabolized in a new pathway *via* indoxyl, N-formylanthranilate, anthranilate, 2,3-dihydroxybenzoic acid (DHBA) and catechol and further degraded by an *ortho* cleavage. Many of the enzymes of the pathway, *viz*., N-formylanthranilate deformylase, anthranilate hydroxylase, DHBA decarboxylase and catechol 1,2-dioxygenase were induced by indole as early as after 5 hours of growth. All these enzymes were demonstrated in cell-free system. The pathway for the degradation of indole in *A. niger* is shown in Fig 4[35]. Our attempts to demonstrate indole oxygenase and indoxyl dioxygenase activities under a variety of conditions were unsuccessful. Indoxyl was unstable and it dimerized in the presence of air to indigo and hence the color of the medium had a blue tinge. This was clearly observed in the

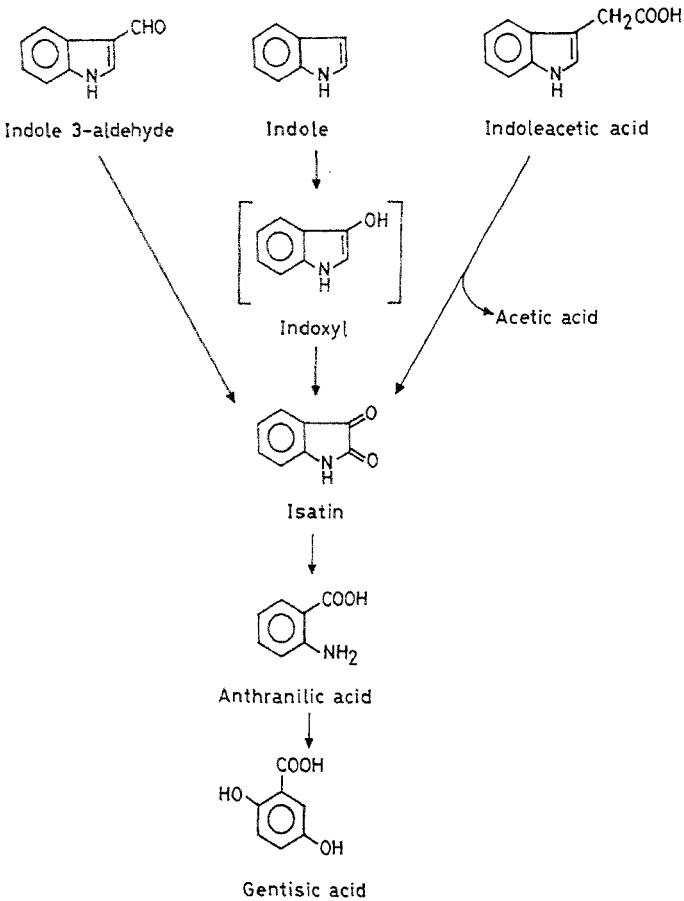

FIG. 3. Catabolism of indole and its derivatives in *Alcaligenes* sp.

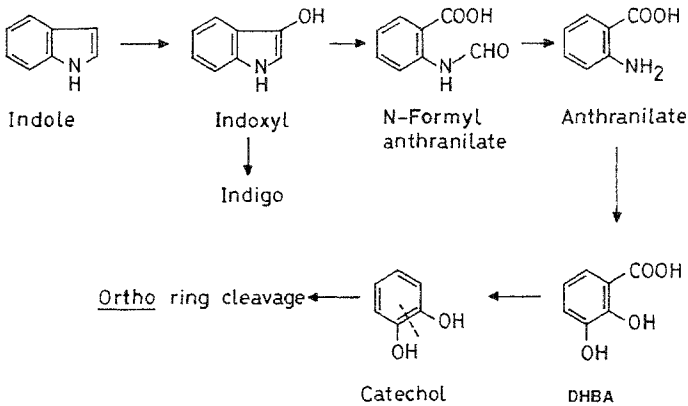

FIG. 4. Proposed pathway for the degradation of indole by *Aspergillus niger*.

immobilization studies when *A. niger* was immobilized on inert matrices such as polyacrylamide, calcium alginate and agar. In these transformations, a good amount of indigo was formed and the medium turned blue in color[34]. The pathway observed in the fungus was different from that reported so far in other systems.

2.2. *Degradation in plants*

Though the metabolism of indole in plants has attracted the attention of several investigators, the exact mechanism by which it is formed is not known. One postulate is that as in microorganisms indole can be formed by the degradation of tryptophan by tryptophanase. This enzyme from *Jasminum grandiflorum* was partially purified and studied in our laboratory[36].

The presence of indole oxidase in plants was demonstrated for the first time in this laboratory from *Tecoma stans*[37,38]. N-Formylaminobenzaldehyde, the ring cleavage product of indole, was identified by trapping it with phenylhydrazine oxalate and was proposed as an intermediate for anthranil formation. Inhibition by salicylaldoxime, diethyldithiocarbamate, its reversal by Cu^{2+} and the requirement of flavin adenine dinucleotide (FAD) for activity suggested the enzyme to be a cuproflavoprotein, unlike tryptophan 2,3-dioxygenase and indole amine 2,3-dioxygenase which are hemo-proteins. On the other hand, indole oxygenase isolated from *Jasminum grandiflorum* yielded anthranilic acid and not anthranil[39] which was the product in *T. stans*[37]. This enzyme in maize gave both anthranil and anthranilate as products[40]. It was a cuproflavoprotein and its activity in parent and hybrid strains of maize was significantly different. By studying tryptophan synthetase and indole oxygenase these authors postulated a possible role for

tryptophan metabolism in the maturation of the developing kernel[41]. However, much of the evidence is circumstantial. Kunapuli and Vaidyanathan[42,43] screened 60 different plants belonging to 33 families for their ability to degrade indole. Of them, only 11 could oxidize indole and there was no correlation between indole degradation and the families to which they belonged. While tecoma, mussaenda and duranta required molecular oxygen for oxidation, eight other species degraded indole anaerobically[43].

Choosing *T. stans* for a detailed study, these authors demonstrated the presence of some indole compounds in the leaf extract[44]. They were identified as indole, skatole, tryptophan and tryptamine. This study revealed a possible presence of indole metabolizing enzymes in leaves of *T. stans*[44]. Indole oxygenase was purified to homogeneity by conventional methods[45] and later by using 5-hydroxyindole coupled to epoxy sepharose[46] and characterized[47]. This enzyme was specific for forming anthranilic acid and no anthranil was formed as the product unlike the maize enzyme[40,41] and indole oxidase[37].

Indole oxygenase from *T. stans* was a new oxygenase as it was not a cuproflavoprotein unlike the earlier enzymes[37–40]. This labile enzyme was mainly found in cytosolic preparations but small amount of activity was also detected in the chloroplast fraction. It had a molecular weight (M_r) of 58,000 and was a monomer unlike the jasmine enzyme[39] which had an M_r of 44,000 and was a dimer. It was insensitive to thiol reagents, atebrin and aminopterin unlike the indole oxidase[37] and jasminum enzyme[39]. Tiron, an Fe^{3+} chelator, drastically inhibited the enzyme suggesting the requirement of Fe^{3+} for indole oxygenase of *T. stans*. The enzyme had broad substrate specificity and oxidized 5-hydroxyindole, 5-bromoindole and 5-methylindole. As the yield of the enzyme obtained by conventional methods was low, affinity chromatography was developed to get a good yield[46]. It was proposed that the C-3 of indole ring should be unsubstituted for the binding of the enzyme.

It is interesting to note that both indole oxidase which forms anthranil[37] and indole oxygenase which forms anthranilate[47] were present in *T. stans*. The reason for the requirement of two enzymes with different properties metabolizing the same substrate is not yet clear. Anthranilic acid, a product of indole oxygenase reaction, was hydroxylated to 3-hydroxyanthranilate which was decarboxylated to *ortho*-aminophenol which was later converted to catechol[48–50]. However, none of these enzyme reactions have been worked out in detail. The fate of anthranil in plants is not known. The scheme for degradation of indole in plants is shown in Fig. 5.

Cauliflower, cabbage and other members of the family *Cruciferae* were shown to induce high level of cytochrome P-450, N-methylaniline-N-demethylase, aminopyrene demethylase, paranitroanisole O-demethylase and arylhydrocarbon hydroxylase activities in rats[51]. Hence phytochemical studies were carried out to identify the inducers of these enzymes in three crucifer vegetables, *viz.*, brussels sprouts, cabbage and cauliflower[52]. The study culminated in the identification of three indole derivatives, namely, indole 3-acetonitrile, indole 3-carbinol and 3,3'-diindolylmethane, as naturally occurring inducers. These compounds are produced during the hydrolysis of indolylmethylglucosinolate by the plant enzyme myrosinase[52]. The pathway delineated for the formation of these indolics is shown in Fig. 6[52].

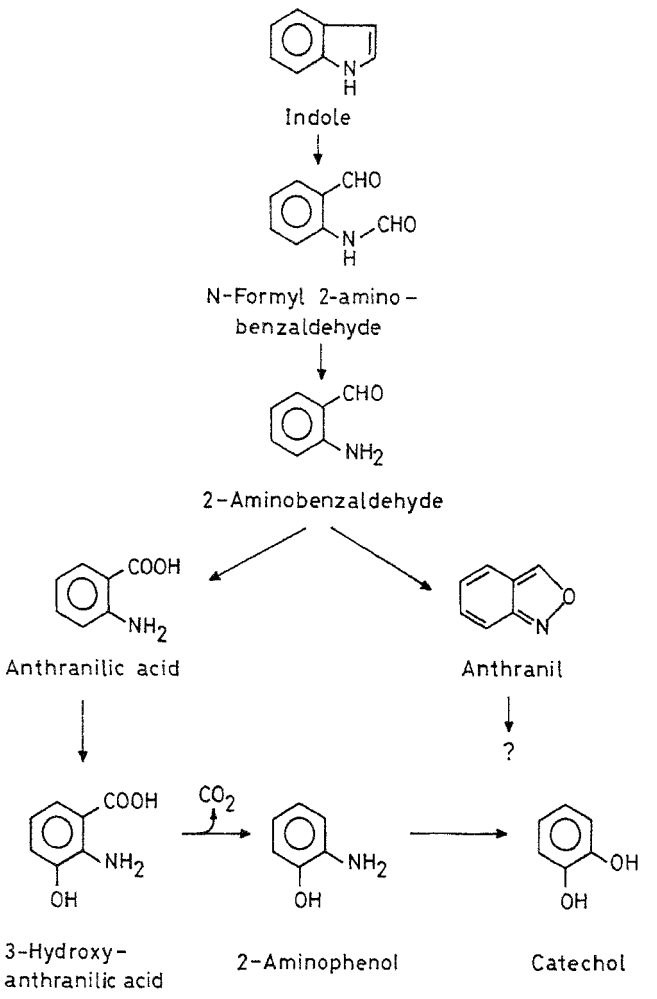

FIG. 5. Metabolism of indole in plants.

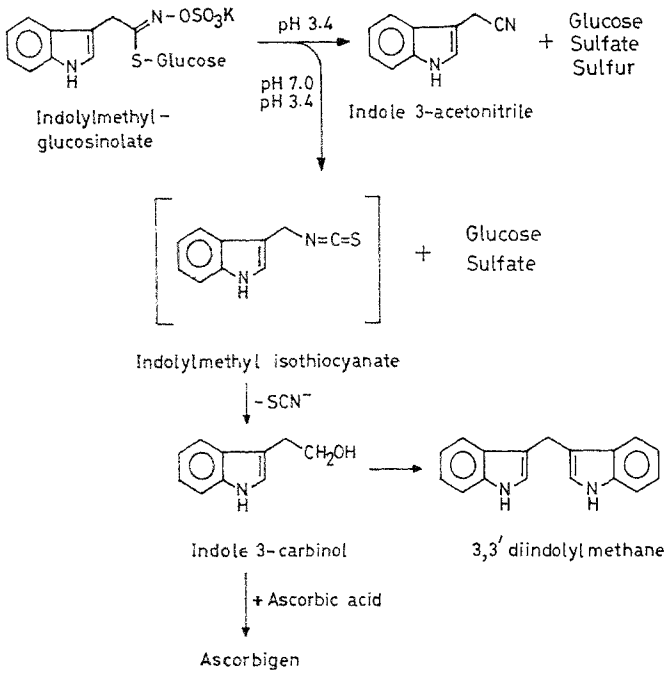

FIG. 6. Degradation pathway for indolylmethylglucosinolate in crucifers.

2.3. *Degradation in animals*

Indole was shown to be toxic to animal systems and it induced many enzymes which could detoxify indole and its derivatives. It has been known for a long time that animals excrete indoxyl as its ethereal sulfate or glucuronide[53]. Williams and coworkers[54,55] studied the fate of indole in rats by feeding them on 2-[^{14}C]-indole. Indole was initially hydroxylated at C-3 position which was reported to be the most reactive carbon[38]. In addition, oxindole (2-hydroxyindole), 5-hydroxy, 6-hydroxy and many hydroxyoxindoles were also formed. All these products were excreted in the urine as sulfate and glucuronide derivatives. The detection of isatin suggested that 2,3-dihydroxyindole could be formed prior to detoxification. It was suggested that oxindole was anaerobically cleaved to *ortho*-aminophenylacetic acid. The pathway for indole degradation in rat is shown in Fig. 7[55].

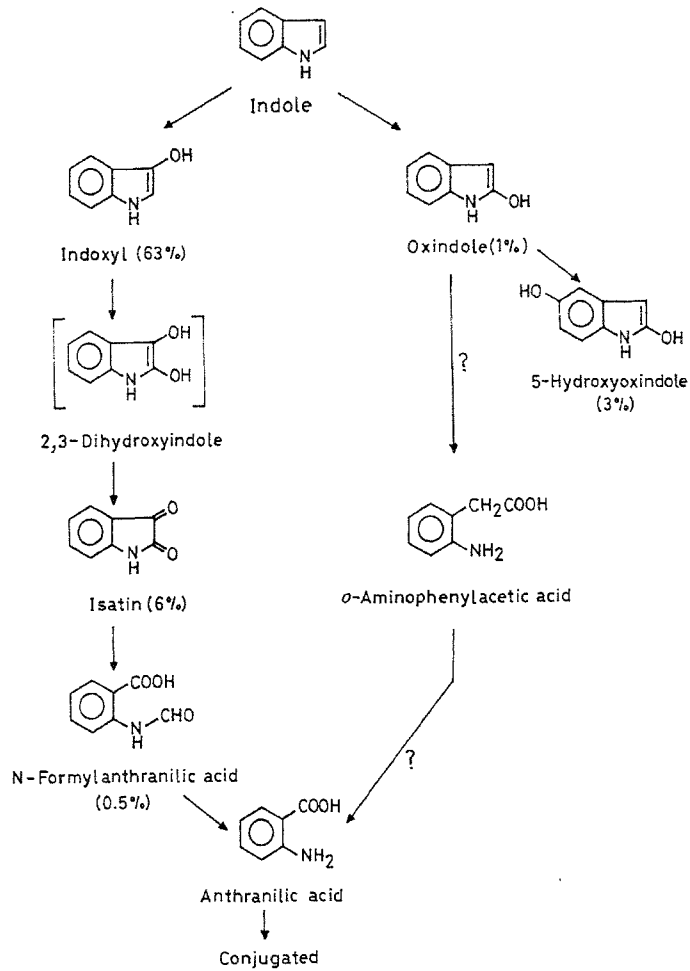

FIG. 7. Proposed pathway for the degradation of indole in rat.

Many species of bugs fed of the floral parts of plants containing significant amount of indoleacetic acid and derivatives degraded these compounds. They were hydroxylated and conjugated with amino acids, sugars and proteins before excretion. Three heteropterans degraded 14 indole derivatives including indoleacetic acid, indole 3-propionate, indole 3-butyrate, indole 5-carboxylate, etc[56]. However, the products of the degradation were not identified.

In humans, indole derivatives are also formed during abnormal metabolism of tryptophan. In East Africans, the gut microflora metabolized tryptophan to indole acrylate and indole 3-propionate which were excreted in the urine as glycine conjugates[57,58]. The excretion of these metabolites in the urine was observed in Hartnup's disease and in normal subjects ingesting 5-hydroxytryptamine present in bananas[57].

It was clearly shown that many hydroxy and methoxy derivatives of indole are found in plants[59-62]. As many as nine 1-methoxyindoles were synthesized in plants which are consumed as food. The fate of these indole derivatives in animals was investigated by Acheson and Nwankwo[63]. In rat, 1-methoxy and 1-acetoxyindole were metabolized similar to free indole, *i.e.*, they were hydroxylated to indoxyl and excreted as sulfate derivatives. Anthranilate was also detected as a product indicating that the indole ring was cleaved. Indole 3-glyoxylic acid and its 1-O-methyl and 1-hydroxy derivatives were excreted essentially unchanged. For the degradation of many other derivatives, reductive methylation was an essential step. The pathways for degradation of some of these compounds are shown in Fig. 8[63].

Although it is evident that these studies on the degradation of indole and its detoxification in microorganisms, plants and animals have resulted in the postulation of diverse pathways, the metabolic schemes have not been proved by isolation of enzymes or by establishing precursor-product relationships.

3. Hydroxylation of indoles

3.1. *In bacteria*

Following the early work of Gray[64], it was established by Sebek and Jager[65] that hydroxylation of indole was an intermediate step in the synthesis of violacein, a dye produced by *Chromobacterium violaceum*. Tryptophan by prior hydroxylation could also serve as the source for the synthesis of this dye. While the hydroxylation in this case occurred at C-5, indole was also hydroxylated at C-3 to give indoxyl, which on nonenzymatic oxidation yielded indigo. None of these reactions could be demonstrated in cell-free extracts. The isolation of 3-hydroxyindole as an intermediate in the formation of indigo was shown in *Pseudomonas indoloxidans*[66]. Indoxyl which was reported to be formed by a direct monohydroxylation of indole seems to be contradictory. The evidence to indicate that double hydroxylations are a common feature in the bacterial metabolism of aromatic compounds[67-69], especially indole, is discussed below.

Indoxyl can be formed by the removal of a molecule of water from 2,3-dihydroxy 2,3-dihydroindole. This was conclusively proved by the work of Gibson and coworkers[70].

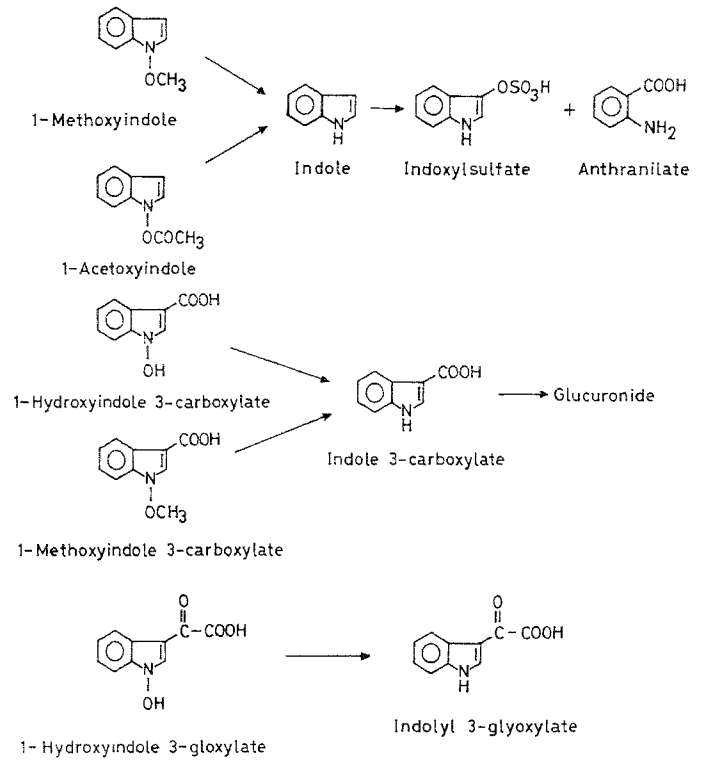

FIG. 8. Major pathways for the detoxification of indole derivatives in rat.

Their experiments showed that the possible mechanism of hydroxylation of indole would be similar to that of anthracene, naphthalene, etc., where a *cis*-1,2-dihydroxy 1,2-dihydrodiol was an essential intermediate. Naphthalene dioxygenase which was cloned in *Escherichia coli*, nonspecifically hydroxylated indole. When *E. coli* was grown in a medium containing tryptophan, naphthalene dioxygenase hydroxylated indole to *cis*-indole 1,2-dihydroxy 1,2-dihydrodiol which after spontaneous elimination of a molecule of water gave indoxyl (Fig. 9)[70]. Indoxyl then dimerized in the presence of air to indigo and hence the medium turned dark blue. The formation of *cis*-1,2-dihydrodiol was also seen in other bacterial

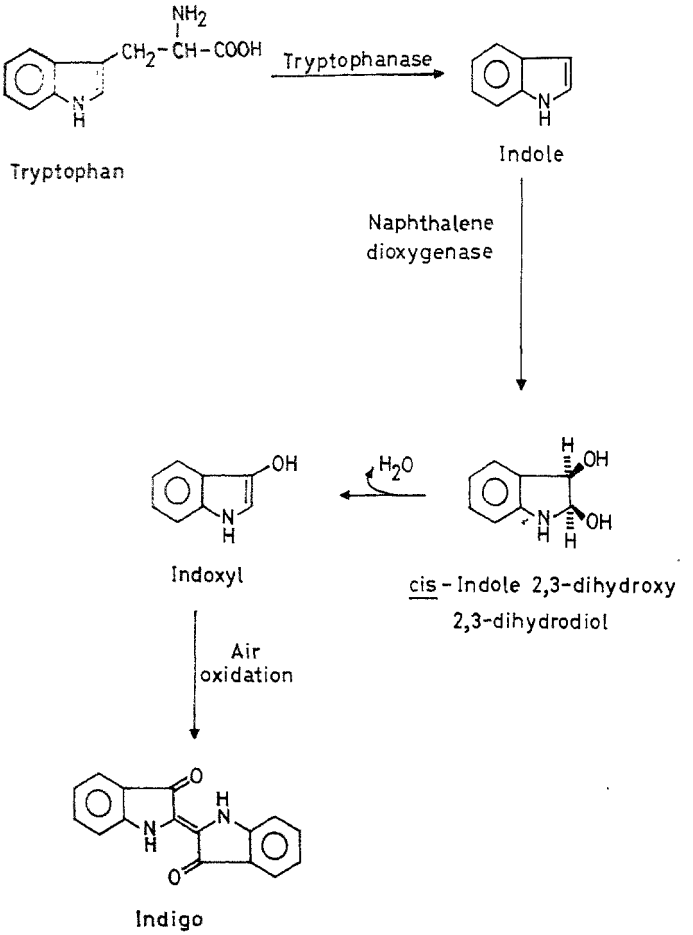

FIG. 9. Proposed pathway for the biosynthesis of indigo in a recombinant strain of *E. Coli*.

dioxygenases. All these organisms thus nonspecifically hydroxylated indole which gave indoxyl and then indigo. The possibility of indole being the intermediate for the formation of indigo was further confirmed by growing *E. coli* in a medium supplemented with indole. Similar observation was made by Clarke and Laverack[71].

Recently, the nonspecificity of these dioxygenases to hydroxylate indole and the subsequent formation of indigo has been exploited to detect some aromatic dioxygenases[72,73]. All these observations support the argument that indoxyl which was proposed as an intermediate in the bacterial metabolism of indole might have arisen by such a mechanism rather than a direct monohydroxylation reaction.

3.2. *In plants*

Corbett and Chipko[74] reported that indole was oxidized by chloroperoxidase in the presence of H_2O_2 to give indoxyl as the major product. Oxindole was the only product formed quantitatively and no other hydroxy derivatives were formed. 2-Methylindole served as a strong inhibitor of chloroperoxidase. Horseradish peroxidase in the presence of H_2O_2 catalyzed hydroxylation of indole to 2,2-*bis*-(3-indolyl)-indoxyl and other products[75]. No reaction was observed when skatole and 3-chloroindole were used as substrates, suggesting the requirement of unsubstituted C-3 of the indole ring for interaction with the enzyme.

Horvath[76] demonstrated hydroxylations of indole using etiolated pumpkin (*Cucurbita pepo*) seedlings. Incubation of these seedlings with indole yielded both 4- and 5-hydroxyindole. Hydroxylation at C-4 was preferred as large amounts of 4-hydroxyindole was formed. Exposure of the seedlings to light for 5 hours reduced the rate of hydroxylation. When the seedlings were grown in light, the unexposed roots were more efficient in hydroxylating at C-4 than the shoots. Infiltration of pedunculate oak and other plant leaves with indole and incubation for a day resulted in the formation of 2,2-*bis*(3-indolyl)-3-indoxyl[77]. Horvath *et al*[78,79] also reported the hydroxylation of the indole ring at C-5 and C-6 positions using plant leaves and stem tissues of *Tridanscantia.*

The hydroxylation of indole as a model substrate was studied in detail by Ishimaru and Yamazaki[80] using microsomes of pea seeds which are known to catalyze hydroxylation of several compounds. An equimolar amount of hydroperoxide was reduced to alcohol during the hydroxylation of indole to indoxyl. A trivial name 'peroxygenase' was proposed for this class of enzymes[80].

It is worth noting that none of these hydroxylating activities has been shown in cell-free extracts so far.

3.3. *In animals*

Dalgliesh *et al*[81] in 1958 showed that in humans the excretion of hydroxyindoles occurred during infection. They isolated sulfate derivatives of few indoles from the urine of man, of which only a skatole derivative was identified. To identify other possible metabolites rats were administered skatole and they detected O-sulfates of indoxyl, 5-hydroxy and

7-hydroxyskatoles in urine. This led to the postulation of a major route for the hydroxylation of skatole in man which was not known till then[81].

The formation of indole and its oxidation to indigo was observed in rabbits and humans[82]. *o*-Aminophenylethanol and other *o*-nitrophenyl derivatives are converted to indole *via* a cyclization mechanism preceding a dehydration. Indole so formed is immediately hydroxylated to indoxyl which is excreted as urinary indican[82]. Occassional occurrence of *bis*-indoles in the urine was observed under many pathological conditions[83]. Many of the indole alkaloids, such as harman, harmaline, etc., are also detoxified in animals after O-demethylation by conjugation[84].

Using rabbit liver microsomes, Ichihara *et al*[85] reported the hydroxylation of indole at C-5 and C-7. They also reported the hydroxylation of indole propionate, indole lactate and indole ethylamine at C-5 and C-7 positions, which specifically required NADPH. However, Udenfriend and coworkers[86] showed 6-hydroxylation in the same system and proved that hydroxylation at C-5 was not possible as reported by the Japanese group. Hydroxylation at C-6 of indole ring was also observed in the case of indoleacetic acid and other indoles[87,88]. Rabbit liver microsomes also hydroxylated indole at C-3 which then gave indigo[89]. This enzymes could not be solubilized. Hydroxylation at C-7[90] and C-5[91] were reported later for indole derivatives.

4. Toxicity and mutagenicity studies on indoles

Indole has a pleasant smell when used at very low concentrations and is used in the preparation of a variety of perfumes and cosmetics; its uses have been discussed by Opdyke[92]. It is also used in confectionaries for the preparation of delicious items including non-alcoholic beverages, ice creams, gelatins, puddings, etc[93].

In recent years, indole and its derivatives have been considered as environmental pollutants. Large amounts of these compounds are released into the nature through industrial effluents and sewage. The development of very sensitive instruments, such as gas chromatography, high-performance liquid chromatography, gas chromatography with mass spectroscopy and other methods has helped in detecting indoles in smoke-polluted environment[12,15], kerosene soot[14], synthetic fuels[13] and in many other sources[16-18,94-96].

4.1. *Toxicity in bacteria*

The toxic effects of indole in *Salmonella typhimurium* was traced to a compound produced by the photoactivation of indole by riboflavin in the medium[97]. It was toxic to other bacteria also[98] and it suppressed cell division in *E. coli* by acting as a bacterial repellent[99]. It also induced the unusual formation of conidia, chlamydospores and high melanin in *Microdochium bolleyi*[100].

Ame's test using histidine requiring *S. typhimurium* strains with and without activation[101] was extensively employed to demonstrate the mutagenicity of indole and its derivatives present in synthetic fuels[13], kerosene soot[14], etc. Studies carried out to relate the structures

Table I
Natural occurrence of some indoles and their mutagenic activities

Compound	Distribution/Occurrence	Revertants/mg
Indole	Coal tar	8,800
	Feces	
	Cigarette smoke	
1-Methylindole	Cigarette smoke	615,000
2-Methylindole	Cigarette smoke	129,000
3-Methylindole (skatole)	Feces	17,900
	Coal tar	
	Cigarette smoke	
Indole 3-ethanol	Microorganisms	2,980
Indole 3-pyruvic acid	Microorganisms	40,800
	Plants	
Indole 3-acrylic acid	Lentil root	2,670
Indole 3-acetonitrile	Cabbage, cauliflower	—
4-Methoxyindole 3-aldehyde	Cabbage, cauliflower	—
4-Chloro 6-methoxyindole	Fava bean	—
4-Methylindole 3-acetonitrile	Crucifers	—
Harman	Cigarette smoke	—
	Wine	

of indoles with their mutagenic nature revealed the role of ring nitrogen[13] and substitution at C-2, C-3, C-5 and C-6 in causing increased mutagenicity[4,9,102]. Indole and many of its derivatives were found in plants and during cooking they became mutagenic due to activation by nitrite, nitrate, hydroxamate, etc., which are either consumed along with food or formed endogenously[6,9,103–108].

The source of some important indoles and their mutagenicity is given in Table I[9]. Most of the indoles found in tobacco and cigarette smoke[12,15] were methyl derivatives[9]. It was shown that 1-methylindole was as potent a carcinogen as benzo(a)pyrene and 3-methylcholanthrene[9]. Indole also enhanced the mutagenicity of tryptophan pyrrolysates[10].

4.2. *Toxicity in plants*

Indole levels near coal-tar industries were high. The plants near these industries developed a mechanism to combat the deleterious effects of indole by producing increased levels of phenolics[109]. Very often the first indication of injury was the increased level of polyphenol oxidase and peroxidase. Indole was more toxic to these plants than quinolines and pyridines, but less toxic compared to quinaldine[109]. The effect of indole(20 μM) in causing decreased pigments in plant cell culture was probably due to the inhibition of pigment formation or repression of enzymes of anthraquinone biosynthesis[110].

4.3. *Toxicity in animals*

High doses of indole administered either orally or intraperitoneally to mice caused

dosage-dependent chromosomal aberrations such as terminal fission, single and pair fragments. There was a direct relation between the level of indole in blood and the frequency of mutation[5]. DNA damage was also observed in the eggs of *Schistosoma* which led to the speculation that the infestation of these eggs might play a role in stomach and colon carcinogenesis[8]. It also caused embryo toxicity in sea urchins[111]. It was suggested that acetylaminofluorene-induced bladder cancers were aggravated by the presence of indole in the diet[11]. There was however a sex dependence for this effect and this was not related to the urinary excretion of N-hydroxy N-acetylaminofluorene. The well-documented correlation between carcinogenesis and mutagenic activities[112] has prompted an uninhibited examination of several indole derivatives used for long-term therapy of chronic illness. Many indole alkaloids such as voacristine caused a dose-dependent cytostatic and cytotoxic effect in eukaryotic cells[113]. Two indole derivatives normally present in diet affected the cell lines of *Xeroderma pigmentosum*[114]. Attempts were made to correlate the excretion of skatole, indole and methane with bowel cancer in man and it has been speculated that there could be a cause and effect relationship between indole-induced DNA damage and colon cancer in these patients[115]. Of the 464 indoles and other compounds isolated from tobacco and tobacco smoke, 25% caused severe damage to the plasma membrane in lung fibroblast cultures suggesting that some of these compounds may not themselves be carcinogenic but could enhance the transport of carcinogenic material found in tobacco into the cells[116].

The presence of a variety of indoles in plants[104,106,107] used as feed for cattle and the formation of indole anaerobically in the rumen[117] prompted an extensive investigation on the toxicity of indole and skatole in ruminants[118,119]. Initial experiments indicated that the toxicity of intraruminally administered tryptophan was related to the anaerobic degradation of tryptophan and the toxic compound was probably skatole. These indoles in cows caused acute pulmonary edema, emphysema[120], hemoglobinuria[117], lung lesions and other effects[117,121,122]. The rumen bacteria especially *Lactobacillus*[123] produced skatole by an inducible system which was repressed in the presence of glucose. Administration of antibiotics effectively counteracted the toxic effects of skatole. Support to this hypothesis was the observation that tryptamine and serotonin which cannot be metabolized to skatole were non-toxic[121]. Lasolacid, an effective drug for the treatment of acute bovine pulmonary edema, reduced the formation of skatole from tryptophan lending support to the view that the formation of skatole was probably an important causative factor in the diesease[120].

One of the major mechanisms of detoxification of indoles is by hydroxylating them by a cytochrome P-450-dependent system and also by induction of demethylase, the function of which is not yet clear. Mostafa *et al*[124] using rat as a model system showed that feeding 1% indole for eight days increased dimethylnitrosamine(DMN) demethylase I and II levels by 262 and 111%, respectively. Administration of 3-methylcholanthrene, phenobarbitone and benzo(a)pyrene caused a decrease in DMN demethylase I activity which acts at low levels of DMN. However, demethylase II was not affected. Since the induction pattern by indole was different from these classical inducers, it was suggested that indole might represent a new class of microsomal inducers, the effect of which differs from those of the classical inducers. Indole also significantly induced arylhydrocarbon hydroxylase(AHH) activity in both rats and hamsters, whereas tryptophan was without any effect[125]. The effect of

aminoacetonitrile and other indole derivatives on the induction of AHH activity was similar to that of indole[126]. The mixed function oxidase-modifying effects of indole compounds illustrate the potential complexity of effects of these dietary constituents on the carcinogenic response[126].

Although the induction of cytochrome P-450 and monooxygenases by indole compounds is well documented, there are reports which question this hypothesis and the discrepancy could be related to the sex and age of the animals used.

Perfusion techniques were carried out to establish the effects of indole on adenylate charge and mitochondrial oxidative phosphorylation activity[127]. Indole lowered the adenylate charge in the small intestine and liver tissues. It also affected the ketone body formation retention in rats. At a concentration lower than 2 mM indole uncoupled the oxidative phosphorylation in mitochondria and higher concentration inhibited the electron transport system[128].

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