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DISTRIBUTION AND METABOLISM OF DOXEPIN*

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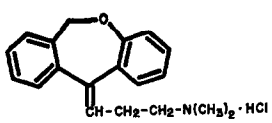
Abstract—Drug metabolism studies with doxepin in rats and dogs reflect the fundamental similarity of its structure to that of the related tricyclic psychotherapeutic agents, amitriptyline and imipramine. Doxepin is well absorbed after oral administration and measurable amounts of doxepin and demethyl doxepin quickly appear in the blood. Although numerous metabolites of doxepin are observed in liver and in urine, only doxepin and demethyl doxepin are found in the rat brain, where the same ratio of *cis*- and *trans*-isomers as in the administered drug is still present. Metabolic transformations include demethylation, *N*-oxidation, hydroxylation and glucuronide formation. In the rat, doxepin and its metabolites are found in all tissues examined but, with the exception of the pigmented eye, are rapidly cleared. This affinity for melanin is also reflected in studies *in vitro* with beef eyeball melanin, where, however, doxepin is less strongly bound than is amitriptyline.

DOXEPIN (Sinequan) (structure I, Fig. 1), a mixture of the *cis*- and *trans*-isomers of 11-(3 dimethylaminopropylidine)-6H-dibenz[b,e]oxepin hydrochloride, is a new drug clinically useful in anxiety and depression.[1]

The *cis*- to *trans*-isomer ratio (resulting from acid-catalyzed equilibration in the final



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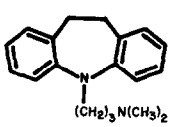

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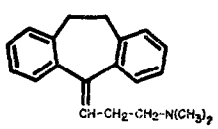

III

FIG. 1. Structural configuration of doxepin (I), imipramine (II) and amitriptyline (III).

* A portion of this work was presented at the 1968 Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Minneapolis, Minn., August, 1968.

step of the synthesis) of the clinical material and of the radioactive material employed in these studies is approximately 15:85.

The investigations to be reported here were undertaken to elucidate the metabolism of doxepin and establish its relationship to that of existing tricyclic psychotherapeutic compounds, particularly imipramine (structure II, Fig. 1) and amitriptyline (structure III, Fig. 1), to which it is chemically and pharmacologically related.

In these studies most of the doses employed (10–30 mg/kg) were those that elicit pharmacological effects in acute animal experiments. It should be noted, however, that these doses are at least 5-fold higher (on a mg/kg basis) than the usual daily human dose. Since previous reports have indicated that some of the tricyclic psychotherapeutic drugs interact with melanin *in vitro* and *in vivo*,[2, 3] and may be responsible for some clinical side effects involving melanin-containing tissues,[4] most of the studies reported here were conducted in Long–Evans (hooded) rats which have pigmented eyes and area of black skin and hair.

MATERIALS AND METHODS

In the experiments described below, doxepin was administered as the hydrochloride salt; in further manipulations the base or salt is implied, as is apparent from the procedures described. Concentrations are expressed in terms of the hydrochloride.

Gas chromatographic assay of doxepin and demethyl doxepin

Heparinized blood was centrifuged and 10 ml of the plasma, made alkaline with 0·6 ml of 1 N NaOH, was extracted with three 10-ml portions of diethyl ether. The combined ether extracts were concentrated to 0·1 ml under nitrogen and, after the addition of 0·1 ml heptane, doxepin and demethyl doxepin were extracted into 0·05 ml of 0·01 N aqueous acetic acid.* Five-μl aliquots of this aqueous solution were injected into a 0·075 in. by 6 ft gas chromatographic column containing 5% W-98 methyl vinyl silicone gum on a support of Gas-Chrom Q, 60–80 mesh, contained in a Perkin–Elmer model 881 dual column gas chromatograph with a flame-ionization detector. Helium carrier gas was used at a flow rate of 30 ml/min; the temperature of the oven was programmed from 200° to 300° at 6°/min. Under these conditions doxepin and demethyl doxepin both had a retention time of 3·7 min in an area free of background peaks. For the differential determination of doxepin and demethyl doxepin, the sample injection was followed immediately by injection of 1 μl of 1% acetic anhydride in diethyl ether.[5] Demethyl doxepin was completely converted to the acyl derivative on the column and the latter appeared with a retention time of 7·8 min in an area also free of background peaks; doxepin was unaffected by this treatment and appeared normally. Under these conditions a sensitivity of approximately 2 ng was obtained; this corresponded to 2 ng/ml of plasma. A standard curve was obtained by injection of 5 μl of various concentrations of doxepin hydrochloride and demethyl doxepin hydrochloride in 0·01 N aqueous acetic acid.

The efficiencies of doxepin and demethyl doxepin recovery were determined by the addition of various amounts of the two materials directly to pooled human serum, followed by extraction and gas chromatographic assay as described above. An average recovery of 90 per cent was observed. These recoveries were used to adjust the

* The substitution of acetic acid for mineral acid resulted in a 50 per cent improvement in sensitivity.

values obtained in the assay of plasma samples. In all experiments blood samples were drawn prior to administration of drug to ensure that no naturally occurring substances were present to interfere in the assay. No such substances were found.

Amitriptyline was administered to one dog for comparative purposes. The gas chromatographic assay for amitriptyline and demethyl amitriptyline was the same as that for doxepin and demethyl doxepin.

Labelled doxepin

Doxepin was prepared from carbonyl-labelled phthalic anhydride by the sequence of reactions shown in Fig. 2.

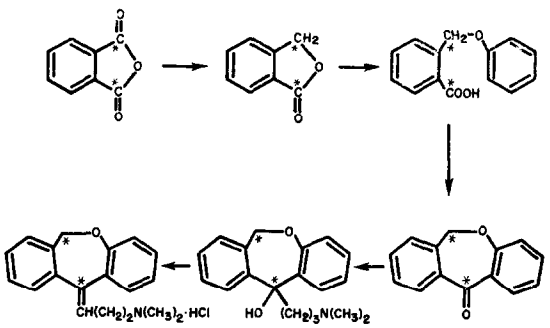

FIG. 2. Reaction sequence for preparation of doxepin.

The product had a specific activity of 3·23 μc/mg. Identity and radiopurity (>99 per cent) were confirmed by thin-layer and paper chromatography in several systems. Chromatography with benzene–chloroform (2:1) on paper saturated with ethylene glycol, a system which separated the *cis*- and *trans*-isomers, revealed the normal 15:85 ratio of components.

Drug administration

Doxepin hydrochloride was dissolved in water, usually at a concentration of 2 mg/ml, and administered to rats via stomach tube or by intraperitoneal injection. Dogs received the drug by stomach tube. Where labelled doxepin was employed, an aliquot of the dose was assayed to determine the actual amount of radioactivity administered.

Sample collection and treatment

Animals were maintained in metabolism cages and excreta collected separately. Feces were homogenized with water, lyophilized and assayed for radioactivity by scintillation spectrometry after digestion with a quaternary ammonium bactericide.* Tissue samples were homogenized with Hyamine and aliquots were assayed (skin samples did not include the hair; stomach contents were washed out prior to homogenization). Urine samples were assayed directly for radioactivity. Blood samples were drawn from the descending aorta of the rat and from the jugular vein of the dog.

* Hyamine, Packard Instrument Co.

Where expired CO_2 was assayed, the rats were kept in sealed metabolism jars which were continuously swept with dry CO_2-free air. Expired CO_2 was trapped in 1 N NaOH and assayed directly.

Chromatographic procedures

Urine samples from rats and dogs were adjusted to pH 11 with NaOH and doxepin and the basic unconjugated metabolites were extracted with methylene chloride. The aqueous phase was incubated at pH 4 overnight with glucuronidase–sulphatase mixture* and subsequently extracted at pH 11 to yield the basic metabolites liberated from their conjugates by enzymatic hydrolysis. Rat brains and liver were homogenized with water and extracted with methylene chloride at an alkaline pH. These extracts and raffinates were chromatographed on silica gel thin-layer plates in comparison with control compounds (doxepin, demethyl doxepin and doxepin-*N*-oxide). The rat brain extracts were also chromatographed on paper to determine the *cis*/*trans*-isomer ratio of the doxepin present. The radioactive materials were localized on the thin-layer plates by autoradiography with Kodak Type KK X-ray film and on paper chromatograms with a Vanguard model 880 Autoscanner. Gibbs' reagent, 2,6-dichloroquinone chlorimide,[6] was also used to delineate doxepin and its metabolites on thin-layer plates.

*Preparation of doxepin-*N*-oxide*

An aqueous solution of doxepin hydrochloride was made alkaline and extracted with methylene chloride. Solvent was removed and the residue, dissolved in methanol, was treated for 5 days with an excess of 30% hydrogen peroxide. Chromatographic examination indicated that the doxepin had been completely replaced by a more polar substance determined from its mass spectrum to be the *N*-oxide.

Melanin binding in vitro

Melanin was isolated from beef eyeballs by differential centrifugation, according to Potts.[7] By a technique essentially identical to his procedure, 2·5 μmoles of drug was incubated with 10 mg melanin granules in 7 ml of pH 4 acetate buffer. The dependence of binding upon the drug/melanin ratio was determined by varying the amount of melanin in the test sample between 2·5 and 10 mg.

RESULTS

Absorption and excretion

The excretion of radioactive material in the urine and feces determined in rats after oral and intraperitoneal administration of doxepin is given in Table 1. The kidney appeared to be the major excretory organ, the urine containing 60 per cent of the radioactivity administered. The similarity in excretory patterns after the two routes of administration indicates that doxepin was rapidly and completely absorbed from the gastrointestinal tract.

The urinary excretion of radioactivity by a dog dosed orally with 100 mg doxepin daily for 5 days is given in Table 2. Approximately 50 per cent of the administered radioactivity was excreted in the urine as compared to 60 per cent in the case of the rat.

* Glusulase, Endo Products, Inc.

TABLE 1. EXCRETION OF RADIOACTIVITY BY HOODED RATS AFTER ADMINISTRATION OF LABELLED DOXEPIN*

Excreta	Time (hr)	Oral dose	Intraperitoneal dose
Urine	0–24	56·76 ± 2·27	55·48 ± 3·78
	24–48	2·22 ± 0·53	2·24 ± 0·94
	48–120	1·14 ± 0·39	0·58 ± 0·15
	Total	60·13 ± 2·24	58·30 ± 4·32
Feces	0–24	24·30 ± 1·59	27·98 ± 6·24
	24–48	1·47 ± 0·33	1·20 ± 0·23
	48–120	0·77 ± 0·24	1·01 ± 0·53
	Total	26·55 ± 1·97	30·21 ± 5·94
Total		86·68 ± 1·96	88·51 ± 4·47

* Four animals per group received 10 mg/kg. Results are expressed as per cent of dose administered (mean ± S.D.).

TABLE 2. URINARY EXCRETION OF RADIOACTIVITY BY A DOG RECEIVING LABELLED DOXEPIN*

Day†	Amount excreted (mg/24 hr)
1	30·6
2	55·4
3	42·9
4	43·6
5	44·3
6	4·0
7	1·7

* Five daily oral doses of 100 mg labelled doxepin were administered to a 10-kg dog. Results are expressed as mg doxepin excreted.
† Day 1 refers to the 24 hr after the first dose; similarly with the other intervals.

TABLE 3. PLASMA CONCENTRATIONS (ng/ml) OF DOXEPIN AND DEMETHYL DOXEPIN IN DOGS AFTER A SINGLE ORAL DOSE OF 100 mg DOXEPIN

	1 hr	3 hr	7 hr
Dog 1 (Male, 10·2 kg)			
Doxepin	<2	34	<2
Demethyl doxepin		4	
Dog 2 (Male, 10·8 kg)			
Doxepin	19	17	6
Demethyl doxepin	2	3	2
Dog 3 (Female, 9·1 kg)			
Doxepin	886	320	69
Demethyl doxepin	54	46	27

Plasma concentrations of doxepin and demethyl doxepin

The concentrations of doxepin and demethyl doxepin in plasma of dogs at various intervals after oral administration of 100 mg doxepin are given in Table 3. The concentrations of both materials reached a maximum at 1–3 hr, doxepin concentrations declining rapidly thereafter.

The effect in dogs of multiple daily doses of 100 mg (approximately 10 mg/kg) doxepin on the plasma concentrations of these two materials was also determined (Table 4). For comparison, another dog received 100 mg amitriptyline daily. The data indicate that doxepin and demethyl doxepin levels had reached a plateau by approximately the second day of administration, the concentrations on the first day being very low (except in dog 3). Amitriptyline levels were somewhat higher than doxepin levels, except on the first day.

TABLE 4. DOXEPIN, AMITRIPTYLINE AND METABOLITES (ng/ml) IN THE PLASMA OF DOGS AFTER FIVE MULTIPLE DAILY DOSES OF DOXEPIN OR AMITRIPTYLINE (100 mg/day)

Drug	Day*					
	1	2	3	4	5	8
Doxepin						
Dog 4 (Female, 10 kg)						
Doxepin	18	33	27	30	25	5
Demethyl doxepin	<2	6	8	12	7	<2
Plasma raffinate†	1770	2030	2600	2130	2740	150
Dog 3 (Female, 9·1 kg)						
Doxepin	320	59	61	38	415	4
Demethyl doxepin	46	46	37	51	99	49
Dog 5 (Female, 11·6 kg)						
Doxepin	11	37	28	162	11	<10
Demethyl doxepin	<3	<3	8	6	3	<3
Amitriptyline						
Dog 3 (Female, 8·7 kg)						
Amitriptyline	3	424	188	206	81	37
Demethyl amitriptyline	<10	80	86	86	54	<10

* Blood sample taken 3 hr after each dose. No drug on days 6–8.
† Determined by radioactivity; others determined by gas chromatographic assay.

Dog 4 received the drug in labelled form so that the amount of non-extractable drug-related material in plasma could be determined. The results indicate that most of the radioactivity in the plasma represented polar metabolites of doxepin, only about 2 per cent being doxepin and demethyl doxepin.

Tissue distribution

The distribution of radioactivity in eleven tissues of the rat at various intervals after a single oral dose of doxepin is given in Table 5. Initially, high levels were noted in liver, kidney, stomach and lung; these declined rapidly. The early high concentrations in stomach tissue are probably associated with the mode of introduction of the drug (stomach tube); after 24 hr, very low levels of radioactivity were seen in the stomach. Radioactivity in the blood and brain was very low. Although appreciable and long-lasting levels of radioactivity were noted in the eyes, concentrations in the dark skin were no higher than those in the unpigmented skin areas. Levels of radioactivity in the eyes of albino rats declined rapidly. A semilogarithmic plot of radioactivity in some tissues vs. time (Fig. 3) indicates multiphasic loss of material. This is consistent with the observed complex pattern of metabolites (see below).

Additional information on the distribution of radioactivity in the rat brain was

TABLE 5. TISSUE LEVELS OF RADIOACTIVITY (EXPRESSED AS μg DOXEPIN/g ± S.E.) IN HOODED RATS AFTER A SINGLE ORAL DOSE OF DOXEPIN (10 mg/kg)

Tissue	1 hr	4 hr	1 day	3 days	8 days	17 days	34 days*	70 days
Blood	0·60±0·13	0·24±0·07	0·03±0·02	<0·005				
Liver	18·72±4·44	6·31±1·48	1·53±0·08	0·68 ±0·01	0·14 ±0·02	0·026±0·001	0·006±0·003	
Liver*†	6·93±0·62	3·85±0·44	0·61±0·09	<0·005				
Kidney	4·11±0·36	2·63±0·63	0·40±0·04	0·16 ±0·01	0·062±0·005	0·023±0·004	0·005±0·002	
Stomach	11·89±1·62	22·04±1·55	0·05±0·01	0·015±0·001	0·006±0·001		<0·005	
Heart	0·82±0·05	0·56±0·09	0·04±0·01	0·015±0·001	<0·005			
Lung	2·12±0·07	0·64±0·23	0·14±0·04	0·030±0·005	<0·005			
Muscle	0·41±0·05	0·31±0·04	0·03±0·01	0·011±0·001	0·007	<0·005		
Eyes	0·60±0·04	0·82±0·12	0·55±0·08	0·41 ±0·02	0·25 ±0·04	0·122±0·008	0·065±0·012	0·027±0·003
Eyes*†	0·50±0·08	0·34±0·11	0·02±0·001	<0·005				
Brain	0·15±0·03	0·09±0·02	0·01±0·001	<0·005	<0·005			
Lt. skin	0·63±0·05	0·56±0·11	0·05±0·01	0·013±0·001	0·006	<0·005		
Dk. skin	0·61±0·05	0·57±0·11	0·05±0·01	0·012±0·002	0·008	<0·005		

* Three animals per group; all others, four animals per group.
† Albino rats.

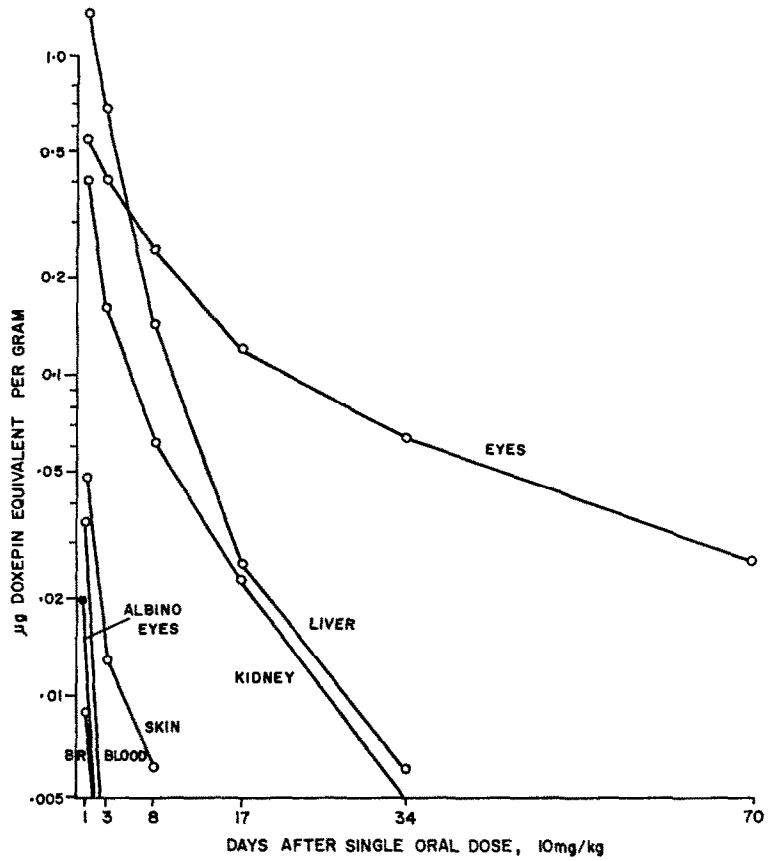

FIG. 3. Tissue levels of radioactivity in the rat after a single oral dose of doxepin (10 mg/kg).

obtained (Table 6); liver and plasma levels were also determined in these animals for comparison. The data indicate that radioactivity was evenly distributed throughout the brain (at this anatomical level), the amounts of radioactivity being appreciably lower than those noted in the livers of the same animals. Chromatographic studies (see below) indicated that the radioactivity in brain consisted primarily of doxepin and demethyl doxepin.

TABLE 6. RADIOACTIVITY (EXPRESSED AS DOXEPIN) IN VARIOUS TISSUES OF THE HOODED RAT 1 hr AFTER A SINGLE I.P. DOSE OF LABELLED DOXEPIN*

Tissue	(μg/g tissue)
Cerebrum	7·19 ± 1·78
Cerebellum	5·18 ± 1·23
Brain stem	6·02 ± 1·57
Liver	20·66 ± 8·29
Plasma	8·38 ± 3·44

* Five animals per group received 30 mg/kg of labelled doxepin. Results are expressed as mean ± S.D.

Interaction of doxepin with melanin

A method for the determination *in vitro* of the affinity of drugs for melanin has been described.[7] By using this method, doxepin was compared with chlorpromazine, known to be highly bound *in vitro* and *in vivo*, and with amitriptyline, which is bound to a low extent *in vitro*. The percentage of each drug bound to melanin is given in Table 7. Under these conditions doxepin is bound to a lesser extent than either of the two comparison drugs. The dependence of binding upon the drug/melanin ratio was also investigated (Fig. 4) by employing various amounts of melanin with a constant amount of chlorpromazine or doxepin. At low melanin/drug ratios, the binding of the two compounds was similar but, as the ratio increased, the differences became apparent.

TABLE 7. PER CENT OF VARIOUS DRUGS BOUND TO BEEF EYEBALL MELANIN*

Drug	% Bound
Doxepin	23·2
Chlorpromazine	57·4
Amitriptyline	38·3

* 2·5 μmoles drug was incubated with 10 mg melanin granules in 7 ml of pH 4 acetate buffer.

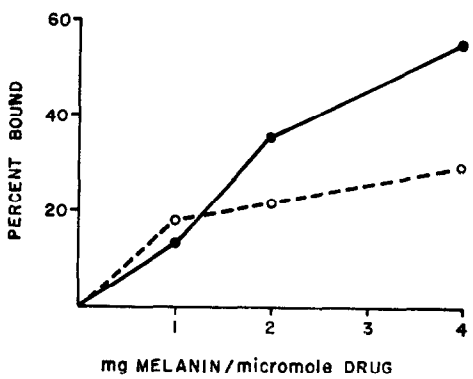

FIG. 4. Per cent of drug bound to melanin at various melanin concentrations. Chlorpromazine, ●—●; doxepin, ○ - - - ○.

Doxepin metabolites

Thin-layer chromatography on silica gel of urine and extracts of urine, brain and liver (Fig. 5) indicated a multiplicity of components. By comparison with chromatograms of authentic samples, the presence of doxepin, demethyl doxepin, and doxepin-*N*-oxide was apparent. An alkaline extract of glucuronidase-treated rat urine yielded, after thick-layer silica gel chromatography and elution, a component which was identified as hydroxy doxepin by mass spectrometry. The molecular weight was 295 with many fragments at 16 mass units higher than corresponding major fragments in a doxepin mass spectrum; since doxepin-*N*-oxide was excluded by comparison with an

authentic specimen, the material must be a hydroxy doxepin. Analysis of the mass spectrum indicated that the hydroxyl group was on one of the aromatic rings, but precise localization was not possible in the absence of appropriate model compounds. Spray reactions, using Gibbs' reagent also suggested the presence of demethyl hydroxy doxepin and didemethyl doxepin.

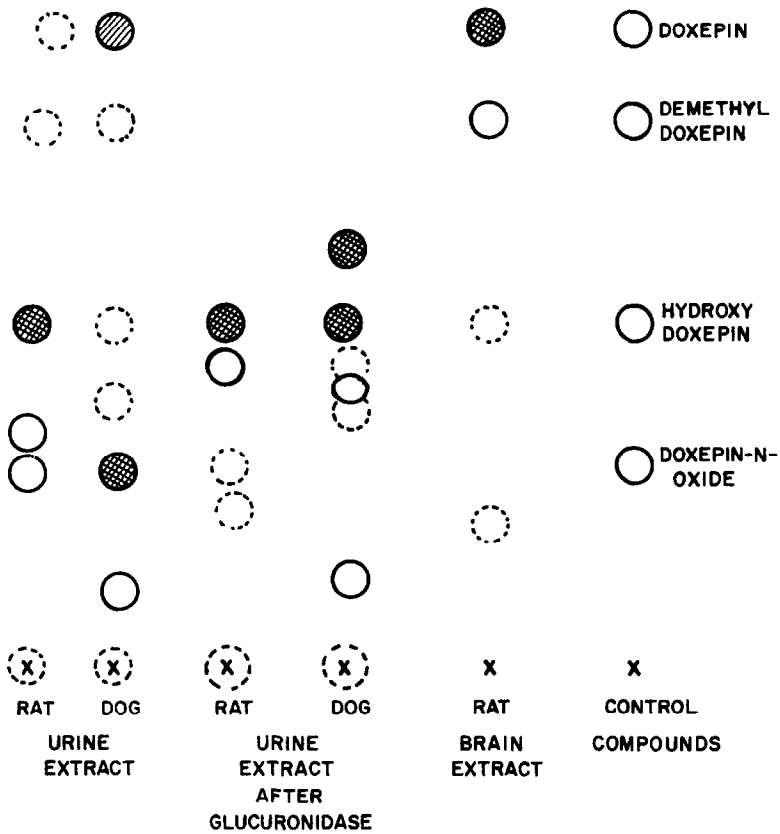

FIG. 5. Thin-layer chromatography of doxepin and metabolites on $CHCl_3$–toluene–methanol–conc. NH_3 (4:3:2:1).

Rat brains, 1 hr after intraperitoneal doxepin administration, were homogenized and extracted with methylene chloride at pH 11. Approximately 90 per cent of the radioactivity appeared in the extract, indicating little if any polar nonextractable metabolites. Chromatography of the extract (Fig. 5) indicated that doxepin and demethyl doxepin were the major substances present. In a system which separated the isomers of doxepin, the normal 15:85 ratio of *cis*- and *trans*-isomers was still present. In contrast to the simple pattern in brain, an extract of liver (75 per cent of the radioactivity was nonextractable) contained numerous metabolites of doxepin. One of the major metabolites present in liver was hydroxy doxepin.

The metabolic patterns are summarized in Table 8. The major metabolites in the rat appear to be hydroxy doxepin and its glucuronide. In the dog, unchanged doxepin

and the glucuronide of hydroxy doxepin predominated. Appreciable amounts of polar metabolites, not extractable into methylene chloride even after glucuronidase treatment, were also present in rat and dog urine. These have not yet been separated or characterized.

There is no evidence that doxepin is extensively metabolized to small fragments which are then incorporated into normal tissue constituents. If this were to occur, some of the ^{14}C from labelled doxepin would be expected to appear as CO_2. Expired CO_2 from three rats receiving 10 mg/kg of labelled doxepin, trapped in alkali, contained no detectable radioactivity.

TABLE 8. DOXEPIN METABOLITES IN DOG URINE AND IN RAT URINE AND TISSUES

Metabolite	RD*		Color‡	Presence†			
	System 1	System 2		Rat urine	Dog urine	Rat liver	Rat brain
Doxepin	1·0	1·0	yellow	+	+++	++	+++
Demethyl doxepin	0·7	0·85	purple	+	+		++
Doxepin-*N*-oxide	0·1	0·35	orange	++	+++	+++	
Didemethyl doxepin		0·43	green	+		++	
Hydroxy doxepin	0·7	0·55	yellow	+++	+	+++	
Hydroxy doxepin glucuronide				+++	+++	+++	
Demethyl hydroxy doxepin		0·48	blue		+		

* Mobility relative to doxepin.
System 1, EtOAc–*N*-methyl pyrrolidine–H_2O (18:3:1);
System 2, $CHCl_3$–toluene–MeOH–conc. NH_3 (4:3:2:1, lower phase).
† Major component, +++; minor component, ++; trace component, +.
‡ Gibbs' reagent: 1% 2,6-dichloroquinone chlorimide in ethanol.

DISCUSSION

In general, the results obtained in metabolic experiments with doxepin in rats and dogs reflect its structural similarity to other tricyclic psychotherapeutic drugs. After labelled doxepin administration, rats excreted 60 per cent of the radioactivity in the urine and dogs excreted 50 per cent. This can be compared with imipramine, where 40–50 per cent appeared over a 4-day period in the urine of rabbits,[8] and with amitriptyline, where 50–60 per cent was excreted in the urine of mice in 24 hr.[9] The close similarities in excretion patterns between rats receiving oral or intraperitoneal doxepin indicate that the drug was well absorbed orally. Imipramine also has been shown[10] to disappear rapidly from the gastrointestinal tract of rats after oral administration.

Although doxepin was well absorbed, concentrations of unchanged drug in blood were extremely low, a consequence of rapid metabolism and distribution of drug and products into other tissue compartments. Similar findings in animals[8, 9, 11, 12] and humans[13] have been reported for imipramine and amitriptyline. Doxepin and demethyl doxepin constituted only about 2 per cent of the total drug-related material present in the plasma of dogs. The nonextractable radioactivity must, therefore, represent polar metabolites. The concentrations of doxepin and demethyl doxepin reached a maximum at approximately 1–3 hr after a single dose and declined rapidly. The plasma concen-

trations of doxepin and demethyl doxepin in dogs receiving five daily 100-mg doses of doxepin were also low, and varied considerably. In two of three dogs, however, doxepin was still detectable in plasma 3 days after the last dose.

Studies in rats with radioactive drug show that doxepin is metabolized rapidly and the products are widely distributed throughout the body, all tissues examined containing measurable amounts of label. The high concentrations of radioactivity in the stomach at 1 and 4 hr are probably a result of the mode of administration of the drug and declined rapidly. Like doxepin, widespread distribution of label has also been seen after radioactive amitriptyline[9] and imipramine,[8, 10, 14] as have the high initial levels in liver, kidney and lung. Doxepin does not appear to give initial concentrations of radioactivity in brain as high as those which have been reported for imipramine[8, 12] and amitriptyline.[9]

There appears to be no differential accumulation of radioactivity in various segments of the rat brain 1 hr after doxepin administration. This finding is in contrast to that of studies of imipramine distribution in the brain of a suicide case,[8] where the drug content of the cerebellum was much lower than that of other areas of the brain. However, this comparison may not be valid since different species were involved and the human dose was extremely high.

The affinity *in vitro* of doxepin for beef eyeball melanin is lower than that of either chlorpromazine or amitriptyline, the latter being the most weakly bound of a large series of tricyclic psychotherapeutic drugs studied earlier.[7] The results obtained with varying amounts of melanin show that the different affinities of doxepin and chlorpromazine for melanin are not observed at high drug to substrate ratios and may indicate that the melanin is saturated under such conditions. Since much lower concentrations of drug are obtained after administration to the intact animal than in this study *in vitro*, a high melanin/drug ratio exists *in vivo*. Here the difference between doxepin and chlorpromazine is most pronounced. The slow removal of radioactivity from the pigmented eye has also been observed with chlorpromazine; studies *in vivo* have implicated a metabolite of chlorpromazine as the species associated with melanin.[7] Studies with pigmented eyes have not been reported for amitriptyline or imipramine. Thiothixene, a thioxanthene tranquilizer, accumulates in the pigmented rat eye, but not in the eye of albino rats.[15] Although doxepin exhibits this same difference in affinities, the differential accumulation in dark vs. light skin observed with thiothixene is not seen with doxepin.

The major routes of metabolism of imipramine and amitriptyline involve hydroxylation on the aromatic rings, hydroxylation on the ethylene bridge, *N*-demethylation, and *N*-oxidation.[16–20] In addition, the side chain can be removed from imipramine;[21, 22] such a reaction has not been reported for amitriptyline where the side chain is joined to carbon (as in doxepin) rather than to nitrogen. A study of the metabolites of doxepin in rats and dogs indicates that many of the above transformations also occur with this drug. Accordingly, doxepin and demethyl doxepin have been found in urine and compared by thin-layer and gas chromatography with authentic samples. The presence of doxepin-*N*-oxide in urine has also been established by thin-layer chromatography in several systems. A hydroxy doxepin was isolated by quantitative thick-layer chromatography and studied by mass spectrometry; the position of hydroxylation could not be established, however. A spray test which distinguishes between secondary and tertiary amines in this type of drug was used to assign tentative

structures to additional doxepin metabolites. A secondary amine, more polar than demethyl doxepin, may be hydroxy demethyl doxepin and another similar substance may be didemethyl doxepin. Since hydroxy doxepin is also obtained from the pre-extracted glucuronidase-treated urine sample, it must also be present as the glucuronide. The metabolic transformations of doxepin are summarized in Fig. 6. The major route in the rat appears to be *N*-oxide formation and hydroxylation; a portion of the hydroxy doxepin is subsequently converted to the glucuronide. In the dog most of the

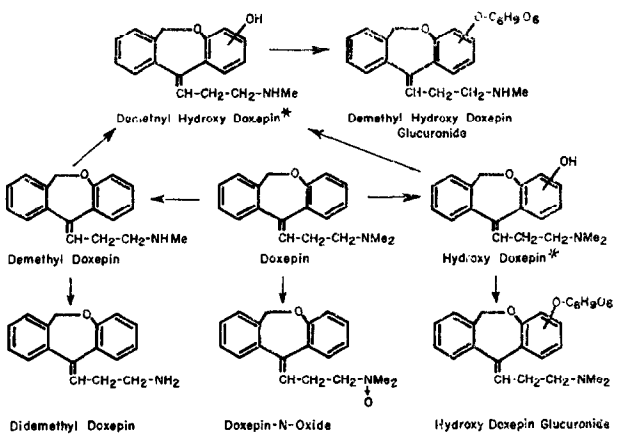

FIG. 6. Doxepin metabolites. *Hydroxylation could also be on other aromatic ring.

hydroxy doxepin is conjugated and appreciable quantities of unchanged drug and *N*-oxide are also excreted. In addition, there are also uncharacterized polar metabolites in the urine of both species.

A wide variety of metabolites, as well as the parent drug, are present in rat liver. In dog plasma, 98 per cent of the radioactivity consists of polar metabolities, all of the extractable material being doxepin and demethyl doxepin. In the brain of the rat, most of the radioactivity is present as doxepin (with no alteration of the isomer ratio) and demethyl doxepin. This is in accord with studies on imipramine[23–25] and amitriptyline[9, 26] in which the parent drug and its demethylated derivative are the only substances found in appreciable amounts. In acute pharmacological experiments comparing doxepin and demethyl doxepin, greater anticholinergic and activity-depressing effects have been noted for doxepin.[27] With respect to these effects, doxepin resembles imipramine and amitriptyline, which are also more active than their demethyl homologues.[28] However, in chronic animal experiments and in clinical investigations, imipramine and amitriptyline are quite similar to their demethylated counterparts.[29] It is likely that doxepin and demethyl doxepin bear the same relationship. Although it has been suggested that the effects of imipramine and amitriptyline are mediated through the demethylated species,[30, 31] there is no evidence that the parent drugs *per se* do not also have the same effects.[29] In this connection it is interesting that human plasma levels of demethyl imipramine and demethyl amitriptyline are almost as high after chronic administration of imipramine and amitriptyline as they are after adminis-

tration of similar amounts of the demethylated drugs.[32] This is reasonable, since it has been shown in experimental animals that demethyl imipramine[33] and demethyl amitriptyline,[34] like their parent compounds, are extensively metabolized and distributed throughout the body. Demethyl doxepin may behave similarly.

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