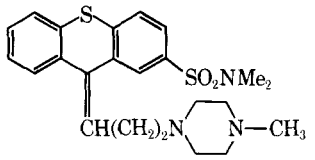
Metabolism of Thiothixene

By D. C. HOBBS

The metabolism of thiothixene, as shown by studies with rats and dogs employing ^{35}S-labeled drug, reflects the fundamental structural similarity of this thioxanthene derivative to other tricyclic psychotherapeutic agents. Like other members of this large group of compounds, it appears to be well absorbed orally, rapidly distributed, and metabolized to a variety of related compounds which are secreted mainly into the bile. The chemical similarity also suggests that it might bind to melanin. Although *in vivo* studies with hooded rats do indeed indicate a preference of thiothixene and/or its metabolites for pigmented over nonpigmented tissues, *in vitro* studies indicate that it is actually one of the least strongly bound of these drugs. In contrast to excreta and other tissues which contain a variety of metabolites, the brain contains only unchanged thiothixene.

THIOTHIXENE (I),[1] the *cis* isomer of *N,N*-dimethyl-9- [3 - (4 - methyl - 1 - piperazinyl)propylidene]-thioxanthene-2-sulfonamide, is a new potent tricyclic psychotherapeutic agent with antipsychotic activity comparable to that of several widely used phenothiazine drugs (1–5).



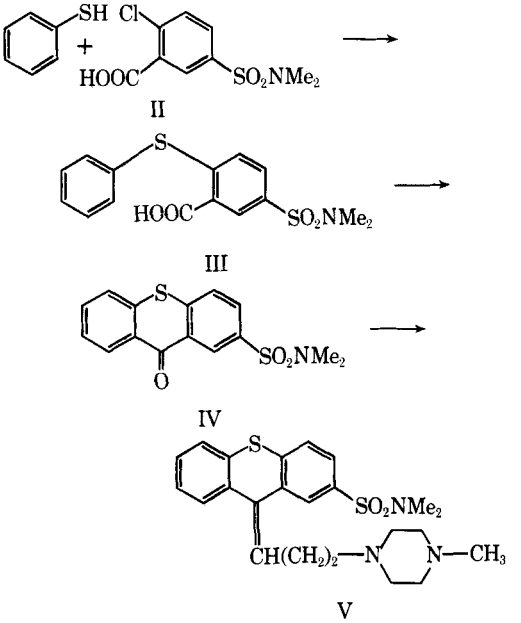
I

The studies to be reported here were undertaken to place the metabolism of thiothixene in perspective with that of existing tricyclic psychotherapeutic agents. In these initial studies using thiothixene labeled with ^{35}S in the nucleus, most of the doses employed (about 8 mg./Kg.) were those which elicit pharmacological effects in acute experiments. It should be noted, however, that these doses are at least ten to twenty-fold higher (on a mg./Kg. basis) than the usual daily human dose.

Since previous reports have indicated that certain tricyclic psychotherapeutic drugs chemically related to thiothixene interact with melanin *in vitro* and *in vivo* (6) and may be responsible for some clinical side effects in melanin-containing tissues (7), hooded rats, which have pigmented eyes and areas of black skin and hair, were included in some of the studies.

METHODS

Labeled Thiothixene—Thiophenol-^{35}S was condensed with 2-chloro-5-dimethylsulfamoylbenzoic acid (II) in alkaline dimethylformamide at 130–140°. The resulting acid (III) underwent ring closure with polyphosphoric acid at 70°. The ketone (IV), in a Wittig reaction with triphenyl-3-(4-methylpiperazinyl)propylphosphonium bromide hydrobromide, yielded V, a mixture of thiothixene and the undesired *trans* isomer. After removal of the latter as the crystalline phosphate, thiothixene-^{35}S was obtained by crystallization of the base from the mother liquor. The initial specific activity of the product was 13.4 mc./mmole. A second lot of material (1.7 mc./mmole) was obtained by dilution of the mother liquors with unlabeled thiothixene, followed by recrystallization. (Scheme I.)



Scheme I

Chromatographic examination of both lots of labeled thiothixene in paper and thin-layer systems established the identity and radiopurity (>98%) of the material.

Drug Administration—Thiothixene base, dissolved in pH 4, 0.25 *M* acetate buffer, was administered to rats and dogs by stomach tube or by intraperitoneal injection. Most of the studies were carried out at a dose level of 8 mg./Kg. However,

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[1] Navane is the registered trademark of Chas. Pfizer & Co., Inc.

TABLE I—EXCRETION OF RADIOACTIVITY[a] BY RATS (AV. WT. 140 GM.) FOLLOWING SINGLE INTRAPERITONEAL OR ORAL DOSES OF 8 mg./Kg. LABELED THIOTHIXENE

	Intraperitoneal		Oral	
	Conditioned[b] (2 Rats)	Unconditioned (4 Rats)	Conditioned[b] (2 Rats)	Unconditioned (4 Rats)
Urine, hr.				
0–24	3.8 (2.6– 4.9)	4.4 (3.1– 5.6)	2.6 (2.6– 2.6)	3.6 (2.8– 5.1)
24–48	0.7 (0.5– 0.8)	3.8 (1.0– 6.4)	0.3 (0.3– 0.3)	1.1 (0.5– 1.5)
48–96	0.3 (0.3– 0.3)	0.5 (0 – 1.3)	0.3 (0.3– 0.3)	0.3 (0.2– 0.3)
Total	4.8 (3.4– 6.0)	8.7 (4.6–11.8)	3.2 (3.2– 3.2)	5.0 (3.5– 6.4)
Feces, hr.				
0–24	43.1 (41.3–44.9)	10.0 (0 –38.7)	73.2 (64.7– 81.7)	45.6 (25.7– 70.4)
24–48	10.9 (8.7–13.1)	21.2 (0.1–72.1)	25.9 (10.8– 41.1)	57.1 (28.3–107.7)
48–96	3.5 (2.3– 4.6)	0.7 (0 – 1.8)	1.7 (0.8– 2.6)	2.8 (0 – 5.1)
Total	57.5 (55.9–59.1)	31.9 (0.5–72.9)	100.8 (78.1–123.6)	105.5 (75.8–133.4)
Total	62.3	40.6[c]	104.0	110.5

[a] Expressed as average (range) percent of dose administered. [b] These rats had previously received 1 mg. unlabeled thiothixene/day for several weeks. [c] Two of the animals in this group were greatly depressed and excreted very little feces, thus decreasing the apparent excretion of radioactivity.

the effects of chronic administration on levels of radioactivity in the liver, eyes, and dark skin of hooded rats were investigated in a group of animals given daily oral doses of 1 mg./Kg. Two animals were sacrificed each second day and the tissues assayed for radioactivity. After 13 days, drug administration was terminated in these animals and in another group of hooded rats which had been dosed simultaneously with 10 mg./Kg. thiothixene. Animals from both groups were then sacrificed at intervals to assess the rate of removal of drug from these tissues.

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.[2] Tissue samples were homogenized with the bactericide and aliquots assayed; skin samples did not include the hair, and stomach contents were washed out prior to homogenization. Urine samples were assayed directly for radioactivity.

Chromatographic Procedures—Urine samples were accumulated from rats receiving daily intraperitoneal doses of unlabeled thiothixene. After treatment with a glucuronidase–sulfatase mixture,[3] an alkaline chloroform extract was prepared and concentrated for thin-layer chromatography. A similar extract was prepared from urine not treated with enzyme. Samples of feces from rats receiving labeled thiothixene by the oral and intraperitoneal routes were extracted before and after glucuronidase treatment at neutral, acid, and alkaline pH and concentrated extracts similarly chromatographed. Bile samples obtained 1–1.5 hr. after administration by these two routes were also included for comparison, as well as a neutral extract of liver. Thin-layer system EDW consisted of ethyl acetate-diethylamine–water (90:15:5) on Silica Gel GF. Areas on the thin-layer strips containing radioactive materials were delineated with a Vanguard model 880 Autoscanner and by autoradiography with Kodak type F X-ray film.

[2] Hyamine, Packard Instrument Co.
[3] Glusulase, Endo Products, Inc.

In Vitro Studies—Melanin was isolated from beef eyeballs by differential centrifugation, according to Potts (6). It was found that a concentration of 5 mg. melanin granules/ml. bound chlorpromazine to the same extent as that reported by Potts for 10 mg./ml. The former concentration was therefore used in the comparative study with 2.5 μmoles compound/ml.

Isolation of Sulfur-Containing Nondrug Related Materials—Twelve rats each received subcutaneous injections of 50 mg. of β-naphthylamine in oil (an amount found sufficient, in preliminary experiments, for the facile recovery of its metabolite) and 1 mg. labeled thiothixene (intraperitoneal) on each of 2 successive days. Urine was collected for a total of 5 days and 2-amino-1-naphthylsulfuric acid isolated by the method of Laidlaw and Young (8).

The method of Dziewiatkowski (9) was used to sample body cysteine in the form of an excreted mercapturic acid. Accordingly, 2 rats each received oral doses of 140 mg. of bromobenzene and intraperitoneal doses of 2 mg. of labeled thiothixene. Crystalline *p*-bromo-phenylmercapturic acid was isolated from the 24-hr. urines as described (9).

Cysteine was isolated (10) from the total carcass protein (except gastrointestinal tract contents) of rats 1 day (bromobenzene animals) and 5 days (β-naphthylamine animals) after administration of labeled thiothixene.

RESULTS

Excretion—Following single intraperitoneal or oral doses of labeled thiothixene, urine and feces were collected and assayed after various intervals (Table I). The route of drug administration did not appear to have an appreciable effect on the route or rate of excretion; the differences in fecal radioactivity were probably artifactual, resulting from the small amount of feces excreted by the depressed animals receiving intraperitoneal thiothixene. Some animals in each group were pretreated with several daily doses of unlabeled drug; these animals excreted the radioactive material earlier than those not pretreated. Whether this was due to saturation of tissue binding sites by the unlabeled material or to induction of drug metabolism cannot be answered by these data.

Most of the radioactivity was excreted in the feces, indicating extensive biliary secretion of thiothixene and/or its metabolites by the rat. That this actually occurred was established by direct measurement of radioactivity in the bile from two rats (Table II) and a dog (Table III) receiving labeled thiothixene. The high levels of radioactivity observed indicate the importance of this route of excretion and the high efficiency of drug absorption following administration by the oral and intraperitoneal routes. The dog excreted a greater fraction of the administered radioactivity *via* the urine than did the rat.

Distribution—Following single doses of labeled thiothixene in the rat, various tissues were removed after 4 or 24 hr. for estimation of tissue radioactivity (Table IV). Four hours after dosing, all tissues examined contained higher levels of radioactivity after the intraperitoneal dose than after the oral dose. However, this may not be a valid index of the relative efficiencies of absorption by these two routes; material absorbed from the intestinal tract goes first to the liver where it appears to be rapidly metabolized and excreted into the bile, whereas drug absorbed from the peritoneal cavity goes first to the general circulation.

At the earlier interval, the highest concentration was seen in the stomach, even after intraperitoneal administration. After 24 hr., however, the liver was the only tissue with appreciable amounts of label. To further investigate levels in the liver, a group of rats receiving single intraperitoneal doses of labeled thiothixene were sacrificed at intervals of up to 28 days; the decline in radioactivity occurred with an early half-life of 3 days and a later half-life of approximately 4.5 days (Table V). Plasma levels of radioactivity in these animals declined more rapidly.

The distribution of radioactivity within the brain of the rat 1 hr. after thiothixene administration was determined, as indicated in Table VI. Small differences were observed, but the ratio of the extreme values was only 1.7. Autoradiography of

TABLE II—BILIARY SECRETION[a] OF RADIOACTIVITY BY THE RAT FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION OF 8 mg./Kg. LABELED THIOTHIXENE

hr.	Intravenous	Oral
0–1/2	0.3	2.5
1/2–1	2.1	7.4
1–1 1/2	4.3	10.3
1 1/2–2	5.8	10.7[b]
2–3	10.0	15.2
3–4	10.5[b]	18.4
4–5	13.3	22.7
5–6	14.9	25.7
6–7	16.2	...
7–22	22.7[c]	30.5
22–30	28.8	43.5
30–48	...	65.0

[a] Cumulative percent of dose administered. [b] A small amount of bile lost from these samples, but there should be little effect on cumulative values. [c] A considerable amount of bile lost from this sample; extrapolation to expected bile flow rate means increasing total 0–30 hr. excretion from 28.8 to 37.1% of the dose administered.

TABLE III—BILIARY AND URINARY EXCRETION[a] OF RADIOACTIVITY BY THE DOG FOLLOWING ORAL ADMINISTRATION OF 14 mg./Kg. LABELED THIOTHIXENE

hr.	Urine	Bile
0–2	...	9.5
2–5	...	23.3
5–22	16.9	46.5
22–29	18.0	48.9
29–46	19.2	51.8

[a] Cumulative percent of dose administered.

TABLE V—LIVER AND PLASMA RADIOACTIVITY LEVELS (mcg. THIOTHIXENE EQUIVALENT/Gm.) IN RATS AFTER SINGLE INTRAPERITONEAL DOSES (8 mg./Kg.) OF LABELED THIOTHIXENE

Time	Liver	Plasma
2 hr.	14.86 (2)[a]	0.93 (2)
6 hr.	9.37 (2)	0.25 (2)
1 day	6.84 (2)	0.06 (2)
2 days	5.88 (2)	0.02 (2)
3 days	3.13 (2)	...
5 days	2.64 (4)	0.01 (2)
7 days	1.84 (2)	...
14 days	0.32 (2)	...
21 days	0.21 (2)	...
28 days	0.06 (2)	...

[a] Number of animals.

TABLE VI—DISTRIBUTION OF RADIOACTIVITY WITHIN THE RAT BRAIN 1 hr. AFTER A SINGLE INTRAPERITONEAL DOSE (10 mg./Kg.) OF LABELED THIOTHIXENE

	mcg. Thiothixene Equivalent/Gm.
Cerebral hemispheres	0.72
Olfactory bulb	0.66
Cerebellum	0.54
Paraflocculus	0.43
Thalamus	0.60
Medulla	0.59
Corpus callosum	0.73

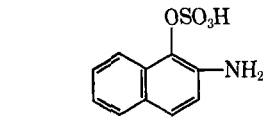
TABLE IV—^{35}S TISSUE LEVELS (mcg. THIOTHIXENE EQUIVALENT/Gm.) IN RATS FOLLOWING A SINGLE DOSE (8 mg./Kg.) OF THIOTHIXENE-^{35}S

	Intraperitoneal		Oral	
	4 hr.	24 hr.	4 hr.	24 hr.
Heart	1.12, 1.50	0.14, 0.09	0.21, 0.13	0.14, 0.05
Lung	4.75, 2.05	0.70, 0.80	0.85, 0.90	0.09, 0.10
Liver	11.43, 7.91	4.42, 5.91	4.96, 7.17	6.60, 5.71
Kidney	1.50, 0.46	0.61, 0.38	0.47, 0.68	0.42, 0.19
Stomach	23.50, 10.69	0.23, 0.46	9.12, 14.35	0.04, 0.05
Skin	1.65, 1.31	0.23, 0.32	0.04, 0.05	0.28, <0.01
Muscle	1.20, 1.25	0.09, 0.14	0.04, 0.09	0.03, 0.02
Brain	0.23, 0.09	0.02, 0.04	0.04, 0.02	0.01, 0.01

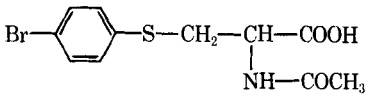
thin slices of brain indicated that more activity was associated with the gray matter than with the white matter.

Since thiothixene is to be administered chronically, tissue levels after multiple doses were investigated (Table VII). Under these conditions, higher and longer-lasting levels were noted in all of the tissues examined, the liver again showing the highest concentrations. Upon chronic administration, as after a single dose, the brain contained the lowest level of radioactivity of these tissues. An additional study was made of levels of radioactivity in liver, eyes, and skin of hooded rats receiving thiothixene in daily oral doses of 1 mg./Kg. [close to that found satisfactory for human clinical use (4)] and 10 mg./Kg. (Fig. 1). Amounts of label in these tissues of the low-dose animals had reached equilibrium by approximately the fifth dose. Following the cessation of drug administration, levels in liver and skin of both high-dose and low-dose animals declined logarithmically with a half-life of approximately 5 days. Levels in the eye remained stationary for several days, then declined at a rate considerably lower than that observed in the liver.

With the exception of the *N*-methyl group(s), fragments from the tricyclic psychotherapeutic drugs do not appear to be incorporated into normal tissue constituents. Some direct evidence on this point for thiothixene was obtained by the isolation of constituents which might contain labeled sulfur derived from thiothixene. Accordingly, 2-amino-1-naphthylsulfuric acid (VI), excreted as a result of the hydroxylation and etherification with sulfate of administered β-naphthylamine, and *p*-bromophenylmercapturic acid (VII), excreted upon condensation



VI



VII

of administered bromobenzene with *N*-acetylcysteine, were isolated. Cysteine, derived from total body protein, was also obtained. The absence of radioactivity in these substances indicates that the sulfur of tissue sulfate and cysteine cannot arise from the ring sulfur of thiothixene.

Interaction of Thiothixene with Melanin—To further assess the relative affinity of thiothixene for melanin-containing tissues as compared to the same tissues without melanin, the tissue levels in the eyes and skin of hooded (pigmented) rats were compared with those in albino animals (Table VIII). The dark skin of the hooded rats contained more label than the white skin of the same animals or the skin of albino rats. Statistical analysis indicated that the difference was significant at the 5% level. Drug concentrations in the pigmented eyes, although comparable to those of the liver at the same interval (Table IV), were nevertheless much higher than those observed in the eyes of the albino rats.

In vitro, the affinity of thiothixene for melanin isolated from beef eyeballs was compared with that of several other tricyclic psychotherapeutic drugs and the *trans* isomer of thiothixene (Table IX). Values for triflupromazine and amitriptylene were quantitatively different from those obtained by Potts (6) in a similar study, although the relative ranking of these two compounds and chlorpromazine was the same. This may have been due to a difference in the potency of the melanin preparations. The extent of binding noted with thiothixene (as well as its *trans* isomer) was similar to that observed with amitriptyline, the least bound of a much larger group of psychotherapeutic drugs investigated by Potts (6).

Metabolites of Thiothixene—Alkaline chloroform extracts of rat urine, before and after treatment with glucuronidase, upon thin-layer chromatography in system EDW, revealed the presence of several UV absorbing spots. There were only traces of material moving in the thiothixene region, but one

TABLE VII—TISSUE LEVELS (mcg. THIOTHIXENE EQUIVALENT/Gm.) IN THE RAT AFTER EIGHT DOSES[a] OF THIOTHIXENE ^{35}-S

	Days After Last Dose		
	1	*2*	*3*
Heart	1.07	0.61	0.81
Lung	2.08	1.67	1.87
Liver	21.35	15.62	12.32
Kidney	3.58	1.77	2.96
Stomach	3.37	3.05	2.84
Muscle	0.98	0.23	0.66
Brain	0.06	0.03	0.21

[a] Three rats (av. wt. 120 Gm.) each received 8 mg./Kg. intraperitoneal thiothixene on days 1, 2, 3, 4, 5, 8, 9, 10.

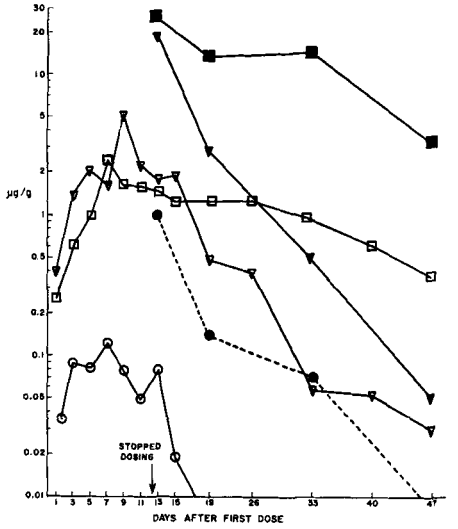


Fig. 1—Levels of radioactivity (expressed as mcg./Gm. thiothixene) in selected tissues of hooded rats during and subsequent to 13 daily oral doses of labeled thiothixene. Key: ■, eyes, 10 mg./Kg.; □, eyes, 1 mg./Kg.; ▼, liver, 10 mg./Kg.; ▽, liver, 1 mg./Kg.; ●, dark skin, 10 mg./Kg.; ○, dark skin, 1 mg./Kg.

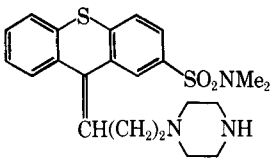
TABLE VIII—TISSUE LEVELS OF ^{35}S (mcg. THIOTHIXENE EQUIVALENT/Gm.) IN SKIN AND EYEBALLS OF HOODED AND ALBINO RATS AFTER A SINGLE INTRAPERITONEAL DOSE OF 8 mg./Kg. THIOTHIXENE-S^{35}

Time →	24 hr.					7 Days	
	Albino		Hooded			Hooded	
Rat No. →	*1*	*2*	*3*	*4*	*5*		*6*
Eyeballs	0.5		12.45		6.69		5.12
Dark skin	...	...	2.36	0.19	0.14		1.01
White skin	0.18	0.10	0.13	0.04	0.06		0.03

TABLE IX—BINDING OF VARIOUS PSYCHOACTIVE DRUGS TO MELANIN GRANULES

	% Bound	
	This Study	Data of Potts
Chlorpromazine	50.4	52
Triflupromazine	44.0	33
Chlorprothixene	52.1	..
Amitriptyline	37	10
Thiothixene	33.5, 33.5	..
P-4657A (*trans* isomer)	36.4	..

of the major metabolites was chromatographically similar to the demethyl derivative (VIII).



VIII

Extracts of feces, bile, and a neutral extract of liver from animals receiving labeled thiothixene were also examined in system EDW (Fig. 2). A large number of metabolites was again observed, including the demethyl derivative, with little or no unchanged thiothixene. All of the bile samples of Table II were chromatographed in the same system with no discernible changes in the pattern of metabolites during the course of the excretion.

Extracts of various portions of the rat brain 1 hr. after thiothixene administration, upon thin-layer chromatography, revealed a single radioactive substance, chromatographically identical to thiothixene. An extract of liver from the same animal showed the usual multiplicity of metabolites.

Extracts of feces (before and after glucuronidase treatment), prepared at neutral, acid, and alkaline pH levels and after heating with strong alkali gave qualitatively similar patterns of metabolites, the data of Fig. 2 being representative.

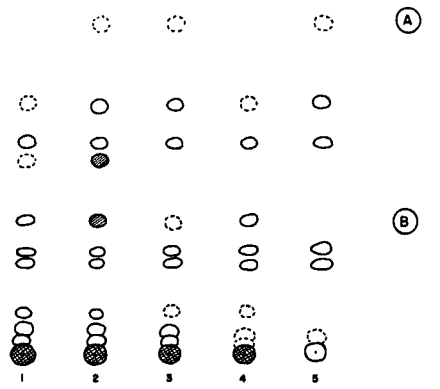


Fig. 2—Thin-layer chromatography of thiothixene metabolites. Key: 1, *bile, i.p. dose;* 2, *bile, oral dose;* 3, *extract of feces, i.p. dose;* 4, *extract of feces, oral dose;* 5, *extract of liver, i.p. dose;* A, *thiothixene;* B, N,N-*dimethyl-9-(3-piperazinylpropylidene)-thioxanthene-2-sulfonamide* (N-*demethyl-thiothixene*).

DISCUSSION

The experimental results reported here indicate that the absorption, distribution, and excretion patterns observed with thiothixene in the rat are generally similar to those of other tricyclic psychotherapeutic agents, as would be expected from the structural relationships involved.

As with other tricyclic psychotherapeutic agents (11–13), thiothixene was rapidly and extensively absorbed following oral administration, very little unchanged drug being recovered in the feces. Further evidence for its prompt assimilation is afforded by the high recovery of radioactivity in the bile of the rat. The low and rapidly diminishing blood levels observed with thiothixene, as with other related drugs (11, 13–16), in the presence of facile absorption indicate rapid excretion and/or distribution into various tissue compartments. The ratio of liver to plasma radioactivity increased with time indicating that the liver is a major depot for storage of thiothixene metabolites. Once absorbed, thiothixene was rapidly metabolized, since bile obtained 0.5 hr. after drug administration apparently contained all of the metabolites noted in later samples.

The relatively large amount of radioactivity excreted *via* the bile is in accord with the structure of thiothixene: comparative studies in rats (17) and dogs (13) with various phenothiazines indicate that biliary secretion is highest for those compounds which contain the 4-methyl-1-piperazinyl side chain. Reabsorption from the intestine (enterohepatic circulation) occurs with many phenothiazines (13, 18–20) and may also be a factor in the disposition of thiothixene. The 5:2 ratio of biliary to urinary excretion after thiothixene administration in the dog is considerably lower than that previously observed with prochlorperazine and trifluoperazine in the dog (13). Nevertheless, the feces still account for more than half of the material excreted. In both dogs and rats, therefore, the liver must play a dominant role in the excretion of thiothixene.

There appear to be no appreciable differences be-

tween the amounts of label excreted in the urine and feces from conditioned animals as compared to those not previously receiving unlabeled thiothixene. The amounts excreted in the feces of the orally dosed animals were greater than those derived from the intraperitoneally dosed animals, but this was probably due to the depressed state of some members of the latter group from which a smaller amount of feces was obtained and therefore does not represent depressed absorption of thiothixene. As discussed above, the biliary secretion was similar by both routes.

Data on tissue levels indicate that thiothixene and its metabolites are spread widely throughout the body, as is the case for the tricyclic psychotherapeutic drugs in general (12, 14, 18). Tissue concentrations in the rat 4 hr. after thiothixene administration were generally higher following intraperitoneal as compared to oral administration; however, at the 24-hr. interval, there were no appreciable differences between levels of radioactivity in the two groups of animals. Concentrations in the lung were lower than those previously noted with chlorpromazine and trifluoperazine (11), prochlorperazine (21), thioridazine (22), and perphenazine (16). The high initial drug levels in the stomach of rats after thiothixene parallel results obtained with chlorpromazine in mice (23) and were presumably a function of the basicity of the two compounds (and their metabolites) coupled with acidity of the gastric mucosa. This phenomenon is not associated with retention of drug within the stomach after oral dosing, since it also occurs upon intraperitoneal administration of thiothixene. Amounts of label in the liver were relatively high and declined at a slower rate than that of the other tissues examined (except the eye of pigmented rats). This long half-life, however, is similar to that observed with several other related drugs (11). Although multiple daily doses of thiothixene resulted in higher and longer-lasting levels in the liver (as well as other tissues), the ^{35}S-concentrations seen in this study were much lower than those achieved with comparable doses of chlorpromazine in cats (24) and prochlorperazine in rats (14, 21).

The low thiothixene levels found in the brain are typical for this type of drug, being noted also with promazine (25), thioridazine (11), trifluoperazine (11), and chlorpromazine (11, 20). The high brain levels of chlorpromazine relative to other tissues, noted by some investigators (24, 26–27) may have been due to methods of extraction and assay which are primarily responsive to unchanged drug. With thiothixene, as with perphenazine (28), chlorpromazine (29), and prochlorperazine (29), only the unchanged drug could be detected upon chromatographic examination of extracts of brain tissue 1 hr. after drug administration. Whether metabolites of thiothixene begin to appear at later intervals was not assessed. The uniform distribution of thiothixene throughout the rat brain at this morphological level has counterparts in studies with thioridazine (11), but differential accumulation has been observed with chlorpromazine (24, 29) and prochlorperazine (29). The higher levels of thiothixene in the gray matter as compared to white matter have also been seen with chlorpromazine (23).

The melanin-containing areas of the rat skin are definite sites of drug retention, having significantly more ^{35}S than unpigmented skin from the same animals or skin from albino rats. However, the pigmented structures of the eye appear to be the areas of highest retention. In the eye, levels approximating those of the liver were rapidly achieved; since the choroid, where the drug presumably accumulates (30), is only 8% of the total wet weight of the eyeball, levels in this structure corrected for more direct comparison with the data of Potts (6) are therefore much higher than those of the liver. Similarly, chlorpromazine and prochlorperazine result in concentrations in pigmented structures up to 100-fold higher than the corresponding liver concentrations (6). *In vitro* studies with thiothixene indicate that its increased psychotherapeutic potency is not reflected in an increased affinity for melanin. In fact, the extent of melanin binding was similar to that of amitriptyline, another nonphenothiazine and the least bound of a larger series of tricyclic psychotherapeutic drugs studied by Potts (6). Since chlorprothixene, another thioxanthene derivative, is highly bound *in vitro* to melanin, the relatively low binding observed with thiothixene may be associated with the dimethylsulfamoyl substituent rather than with a change in the nucleus from phenothiazine to thioxanthene. Nevertheless, the results here indicate that drugs bearing the thioxanthene nucleus behave qualitatively as do their phenothiazine counterparts.

Rapid equilibration of drug levels within the eye and liver was indicated in experiments involving daily oral administration of thiothixene. Concentrations of radioactivity stopped rising after only 5 days. The eye and liver tissue levels attained with doses of 10 mg./Kg. were approximately 10 times those achieved after 1 mg./Kg., indicating no impairment of absorption, distribution, or metabolism within this range. Following withdrawal, levels began to decline immediately in the liver and skin, but there was a lag phase in the eye following which label was removed at a rate slower than that in the liver. The absence of a similar lag in the removal of label from skin (where it is presumably also bound to melanin) may indicate a difference in the nature of the complex. This may be due to differences in the chemical nature of the thiothixene metabolite interacting with the melanin, or in further transformations in the complex once formed. It has been suggested (31) that 7-hydroxychlorpromazine or a further metabolite thereof is the active species involved in chlorpromazine pigmentation; a similar thiothixene derivative may conceivably be involved.

As with the related tricyclic drugs, thiothixene was rapidly metabolized to a wide variety of derivatives with little of the unchanged drug being recovered. The liver appears to be the major site of metabolism, since bile samples obtained within 0.5 hr. of drug administration contained as great a diversity of metabolites as urine and bile samples taken at later intervals. The only metabolite of thiothixene yet tentatively identified is the *N*-demethyl derivative; such demethylation on the side chain is a common feature in the metabolism of drugs of this type.

Although inorganic sulfate has been implicated as a metabolite of ^{35}S-labeled chlorpromazine (26), indicating fission of the ring system, later studies (32) with chlorpromazine and promazine revealed no

evidence for such a reaction. Ten percent of the sulfur of phenothiazine, but an insignificant amount from promazine, was excreted as inorganic sulfate by dogs (25). There have been no subsequent reports concerning the derivation of sulfate from the tricyclic psychotherapeutic agents. Since the thioxanthene nucleus may behave in a manner unlike that of the related phenothiazine drugs, direct evidence on the ultimate fate of the nuclear sulfur was sought. Cysteine has been shown to be derived, in part, from inorganic sulfate (33) and was therefore felt to be a reliable indicator of sulfur metabolism. Since a mercapturic acid derived from administered bromobenzene and cysteine, an ethereal sulfate resulting from detoxication of administered β-naphthylamine, and tissue protein cysteine were all devoid of measurable radioactivity following the administration of a single high dose of thiothixene-^{35}S, it can therefore be reasonably concluded that the nuclear sulfur of thiothixene cannot enter the body sulfur pool. This would also tend to indicate that the nucleus remains intact, as has been concluded for the phenothiazine drugs.

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Keyphrases

Thiothixene metabolism
^{35}S-Labeled thiothixene—synthesis
Tissue distribution thiothixene
Thiothixene melanin interaction
Thiothixene excretion
Scintillation spectrometry—analysis
TLC analysis of urine
Thiothixene metabolites