was also described by Youdim and Sandler,[2] who found that the increase was greater in heart than in brain or liver. Similar results were obtained with Diazo-ICA.

An increase in membrane permeability was proposed by Izumi *et al.*[1] to explain the activation of MAO in mitochondria after reserpine administration, although a more direct chemical action of reserpine on the enzyme itself may also be possible. It appears that a permeability change may be involved to some extent in activation of a tissue homogenate by Diazo-ICA, because the increase in activity caused by Diazo-ICA was somewhat less after sonication of the tissue homogenates.

In the presence of calcium, activation was complete and EDTA reversed only calcium-potentiated activity, although calcium alone had no effect on the MAO activity of a whole homogenate of liver.

Previous studies showed that Diazo-ICA caused chemical modification of the sulfhydryl groups of mercapto compounds and biological sulfhydryl groups.[4] Mercaptoamino compounds such as cysteine or cysteamine were found to prevent activation of the enzyme activity by Diazo-ICA completely and preliminary treatment of MAO with iproniazid also prevented activation of the MAO by Diazo-ICA.

Gokin and Krivchenkova[9] found that various mercaptoamino compounds inhibit rat liver mitochondrial MAO activity and that preliminary treatment of the enzyme with cysteamine prevented the irreversible inhibition of the enzyme activity induced by iproniazid to a significant extent.

These findings indicate that Diazo-ICA may interact with the active center of MAO attacked by iproniazid or mercaptoamino compounds.

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Human metabolism of orally administered pentazocine

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AN EARLIER communication[1] has described the metabolism *in vitro* and *in vivo* of pentazocine in the monkey and the metabolism *in vitro* of this drug in the mouse and rat. It was found that either of the methyl groups of the dimethylallyl side-chain of pentazocine could be hydroxylated and that one of

the resulting alcohols could be further oxidized to the corresponding carboxylic acid. This communication reports the metabolism of pentazocine in man.

Pentazocine, its "*trans*"-alcohol and "*trans*"-acid were available in the author's laboratory.[1] Amberlite XAD-2 synthetic resin was obtained from Rohm and Haas, Inc., Philadelphia, Pa., and cleaned by washing thoroughly in tap water on a U. S. Series No. 40 wire screen, and then by repeatedly and serially suspending the resin in methanol, acetone, methanol and, finally, water. One × 10 cm beds of the resin in water were packed in short-glass columns equipped with a 25-ml reservoir and a Teflon stopcock.

Free plus conjugated pentazocine and metabolites were determined as follows: A 5-ml sample of urine was diluted with 20 ml of 0·1 N, pH 5·5 acetate buffer, 0·05 ml of $CHCl_3$ and 0·125 ml of Glusulase (Endo Labs., Inc., containing approximately 150,000 units of β-glucuronidase and 40,000 units of phenol sulfatase per ml) was added, and the mixture was incubated under a nitrogen atmosphere at 37° for 3 days. The samples then were made acid (pH 2–5) with a drop of concentrated HCl and passed through 1 × 10 cm beds of Amberlite XAD-2 synthetic resin (Rohm and Haas, Inc.) at a rate of about 1 ml per min. The columns were washed twice with 20 ml of water and allowed to drain. Then the columns were eluted once with 10 ml of methanol and twice with 20 ml of methanol. The combined methanol eluates were taken to dryness on a steam bath and under a stream of air. The residues were dissolved in a minimum volume of methanol and were transferred quantitatively with a minimum volume of methanol to small centrifuge tubes, taken to dryness and re-dissolved in 1·00 ml of methanol. Fifty to 500 μl of the resulting solutions were transferred to 2-ml centrifuge tubes and reduced to dryness. Exactly 100 μl of a 0·02% (w/v) solution of tetracosane in hexane was added to each tube as an internal standard and also reduced to dryness. One hundred μl of DMF Sil-Prep (Applied Science Labs.) then was added and the tubes closed with a tightly fitting polyethylene stopper. When all the residue was dissolved, the sample was ready for gas–liquid chromatography. Fresh standards of drug and metabolites were prepared by drying samples of their solutions in methanol in 2-ml centrifuge tubes and proceeding as with urine samples.

Gas–liquid chromatography was performed isothermally at 225° using a Varian Model 2100 gas chromatograph equipped with a flame ionization detector and using 6-ft, 2-mm i.d. glass columns packed with 3% OV-1 Gas Chrom Q (Applied Science Labs.). The carrier gas was nitrogen. One-μl samples were injected, using a 2-μl ethyl acetate flush to insure quantitative injection. Sample concentrations were adjusted when necessary so that all standards and samples could be run at the same electrometer settings. Standard curves were prepared by plotting the ratios of the peak heights or peak areas (peak height × the ½-height width) of drug standards to the peak height of the internal standard (tetracosane) against the concentrations of drug standards.

The excretion of pentazocine and its metabolites was followed in the urine of four male volunteers who took two different oral formulations—Formulation I tablets containing 56·5 mg pentazocine·HCl and inert fillers, and Formulation II tablets containing 56·5 mg pentazocine·HCl, 390 mg aspirin, and inert fillers—in a crossover design on 2 consecutive weeks. Urine samples were taken at 0, 2, 4, 8 and 24 hr after administration of the drug and preserved frozen until analyzed.

The urines of volunteers receiving 50-mg doses of pentazocine in the two oral formulations were analyzed for free plus conjugated pentazocine and metabolites. Pentazocine and its "*cis*"-alcohol and "*trans*"-acid metabolites, as previously identified in the monkey, were identified by the relative retention times of their trimethylsilyl-derivatives as compared to docosane.[1] No evidence for the presence of the "*trans*"-alcohol, found as a metabolite in monkey urine, was obtained; presumably in man, oxidation to the corresponding "*trans*"-acid occurs too rapidly to allow escape of the alcohol into the urine.

The excretion patterns of the three compounds found are reported in Table 1. Since an authentic standard of crystalline "*cis*"-alcohol was not available, amounts of it in the urine were estimated by comparing the ratio of the area of its peak and the peak height of the internal standard to the ratios of the peak areas of known amounts of its isomer, the "*trans*"-alcohol, and the peak height of the internal standard. The excretion patterns of different individuals taking either formulation are similar and the average 24-hr recoveries of all three compounds are similar when the two formulations are compared. The excretion rates of pentazocine and its metabolites each reached a maximum between the second and fourth hours after administration of the drug (Fig. 1). These maxima correspond roughly to the time of maximum levels of pentazocine in plasma after oral administration.[2] The rate of excretion of free pentazocine into the urine after oral administration of the drug appears to have a

TABLE 1. URINARY EXCRETION OF PENTAZOCINE AND METABOLITES IN FOUR MALE VOLUNTEERS TAKING TWO ORAL FORMULATIONS OF PENTAZOCINE*

Subject	Collection period (hr after dose)	Per cent of 56·5 mg dose of pentazocine · HCl found as: Pentazocine Formulation I	Pentazocine Formulation II	"*cis*"-Alcohol Formulation I	"*cis*"-Alcohol Formulation II	"*trans*"-Acid Formulation I	"*trans*"-Acid Formulation II
1	0–2	4·0	3·9	2·3	2·7	11·7	9·1
2		3·2	1·8	2·2	1·3	8·8	7·8
3		3·4	3·4	2·2	2·4	11·5	12·3
4		1·4	1·9	1·2	1·9	5·4	6·4
Av.		3·0	2·8	2·0	2·1	9·3	8·9
1	2–4	2·7	2·9	2·4	3·1	10·8	10·3
2		3·2	3·4	2·6	1·5	11·6	11·5
3		3·1	2·8	2·8	2·8	13·1	12·8
4		3·2	4·0	2·6	4·1	10·8	15·5
Av.		3·0	3·3	2·6	2·9	11·6	12·5
1	4–8	2·3	2·4	4·4	4·9	9·6	9·6
2		3·3	3·4	2·0	3·1	7·9	14·3
3		2·2	2·0	3·1	2·5	13·6	12·2
4		3·1	2·6	6·1	4·4	16·4	13·1
Av.		2·7	2·3	3·9	3·7	11·9	12·3
1	8–24	0·3	1·1	4·5	3·9	5·5	12·0
2		1·2	1·5	3·1	2·1	7·3	7·1
3		0·5	0·7	2·2	1·6	7·9	6·5
4		1·0		2·5		3·8	
Av.		0·8	1·1	3·1	2·5	6·1	8·5
Overall average:		9·5	9·5	11·6	11·2	38·9	42·3

* Formulation I contained 56·5 mg pentazocine · HCl and inert fillers; Formulation II contained 56·5 mg pentazocine · HCl, 390 mg aspirin and inert fillers.

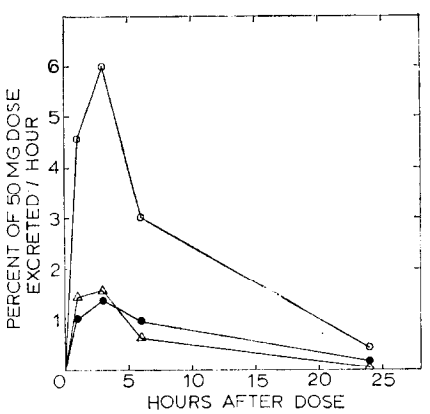

FIG. 1. The average rates of excretion of pentazocine and metabolites into the urine of four male subjects, taking 56·5 mg of pentazocine·HCl in two oral formulations in a crossover experiment. △ = pentazocine. ● = "*cis*"-alcohol metabolite. ○ = "*trans*"-acid metabolite.

somewhat earlier maximum near 1½ hr.[3] The average recovery of the dose in 24 hr in the urine of all subjects was 61·5 per cent. These overall recoveries compare closely to the overall recovery in the urines of four subjects in 24 hr of 62·8 per cent of the radioactivity in i.m. doses of tritium-labeled pentazocine.[4]

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Modification of external sulfhydryl groups of Ehrlich ascites tumor cells with 6,6′-dithiodinicotinic acid*

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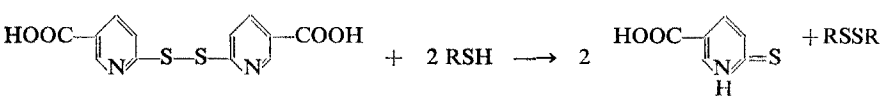
CERTAIN disulfides react with thiols in an irreversible manner, as exemplified for 6,6′-dithiodinicotinic acid (CPDS):[1]

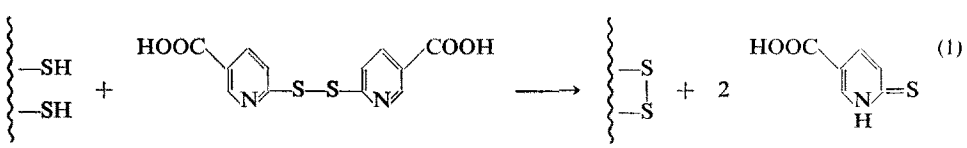


CPDS 6MNA

The fact that 6-mercaptonicotinic acid (6MNA) is virtually completely in the thione form prevents further interaction of this compound with disulfides. We have studied many other "thione-forming" disulfides, belonging to various heterocyclic systems, and found that they all react essentially irreversibly with thiols.[2] The shift in wavelength of the absorption maxima in the ultraviolet and visible range due to formation of the thione has been used as the basis of a method for the determination of thiols.[3]

Enzyme-catalyzed interactions occurring in living cells and leading to formation of 6MNA from CPDS have also been studied.[1] It has been shown that CPDS reacts with Ehrlich ascites (EA) cells to a lesser extent when these cells are intact than when they are broken.[1]

In the case of tissues, two possible reactions can be written between CPDS and cell thiols:



(Type A) (1)

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