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SHORT COMMUNICATIONS

The metabolism of ^{35}S-labeled perphenazine (Trilafon)*

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THE DISTRIBUTION and excretion of total radioactivity, resulting from administration of ^{35}S-labeled perphenazine, has been studied previously.[1] This report deals with the chemical nature of the radioactive material of brain, liver, and urine, as a function of time, after the administration of labeled perphenazine.

METHODS

Male rats from the Charles River Breeding Laboratory were used; they weighed approximately 100 g. The standard dose of perphenazine dihydrochloride was 0·6 mg/kg body weight given by subcutaneous injection. The animals were housed in individual metabolism cages and urine was collected for radioanalysis. At appropriate times after injection, groups consisting of 3 animals were sacrificed. Each whole brain or liver was quickly homogenized at 4 °C and immediately lyophilized. An aliquot of each lyophilized tissue homogenate was placed in a Soxlet thimble and continuously extracted with absolute methanol for 16 hr. The amount of radioactivity in the methanolic extract and the residual powder was then determined.[1] Aliquots of the lyophilized samples were also analyzed prior to extraction in order to calculate the recovery.

Small aliquots of urine were applied directly to Whatman No. 1 paper for chromatography, while methanolic tissue extracts were concentrated under a stream of nitrogen before application. Carrier perphenazine and perphenazine sulfoxide were routinely spotted on the paper and were visualized by spraying the developed chromatographic strips with Dragendorff's reagent.[2] Ascending paper chromatography, using *tert*-amyl alcohol : 5% acetic acid, was employed for 16 hr. In this system perphenazine and perphenazine sulfoxide had R_f values of 0·73 and 0·48, respectively. Scanning for radioactivity was carried out in a gas-flow strip counter.

The preparation of liver homogenate and its fractionation were performed according to Hogeboom,[3] with a slight modification in which only 0·25 M sucrose was used for the isolation of the nuclear fraction. Brain tissue was fractionated according to procedure II of Brody and Bain,[4] but omitting centrifugation at 800 g. Aliquots of the original homogenate and of each fraction were oxidized and counted in the usual way.[1]

It ought to be mentioned that perphenazine in solution is very sensitive to ultraviolet light. When compared with chlorpromazine under conditions described by Forrest *et al.*,[5] perphenazine after 1 hr of irradiation already has been totally converted to a material(s) remaining near the chromatographic origin, while appreciable amounts of chlorpromazine remained unaltered after 6 hr.

RESULTS AND DISCUSSION

The chromatographic pattern of radioactivity in rat urine is illustrated in Fig. 1. The difference in the concentration of perphenazine metabolites with respect to time is quite apparent. Initially, the sulfoxide represents the major urinary metabolite, but at later time intervals other substances appear in increasing number and relative concentration. In all cases there seems to be a complete absence of perphenazine.

The continuous methanol extraction of perphenazine or its metabolites, or both, from lyophilized tissue homogenates has been selected over many other procedures tested. The results from liver and

* Trilafon is the registered trademark of Schering Corporation.

brain tissues are summarized in Table 1. The quantitative recovery of the radioactivity from brain at all time-intervals is in sharp contrast to the increasingly lower yields obtained from liver as time progresses. Chromatographic analysis of methanolic extracts from brain taken 1 and 4 hr after administration of ^{35}S-labeled perphenazine indicated the presence of a single radioactive spot corresponding to perphenazine carrier, while similar extracts from the liver after 1 hr showed a

TABLE 1. CONTINUOUS METHANOL EXTRACTION OF RADIOACTIVE MATERIAL FROM TISSUE AT VARIOUS TIME INTERVALS AFTER INJECTION OF ^{35}S-LABELED PERPHENAZINE

Time, hr	% Extracted	
	Liver	Brain
1	84·9 ± 2·3*	98·7 ± 0·1
4	71·3 ± 3·5	98·9 ± 0·2
24	43·5 ± 6·4	96·9 ± 0·2
Control	99·6	99·6

* Mean from 3 samples ± S. E.

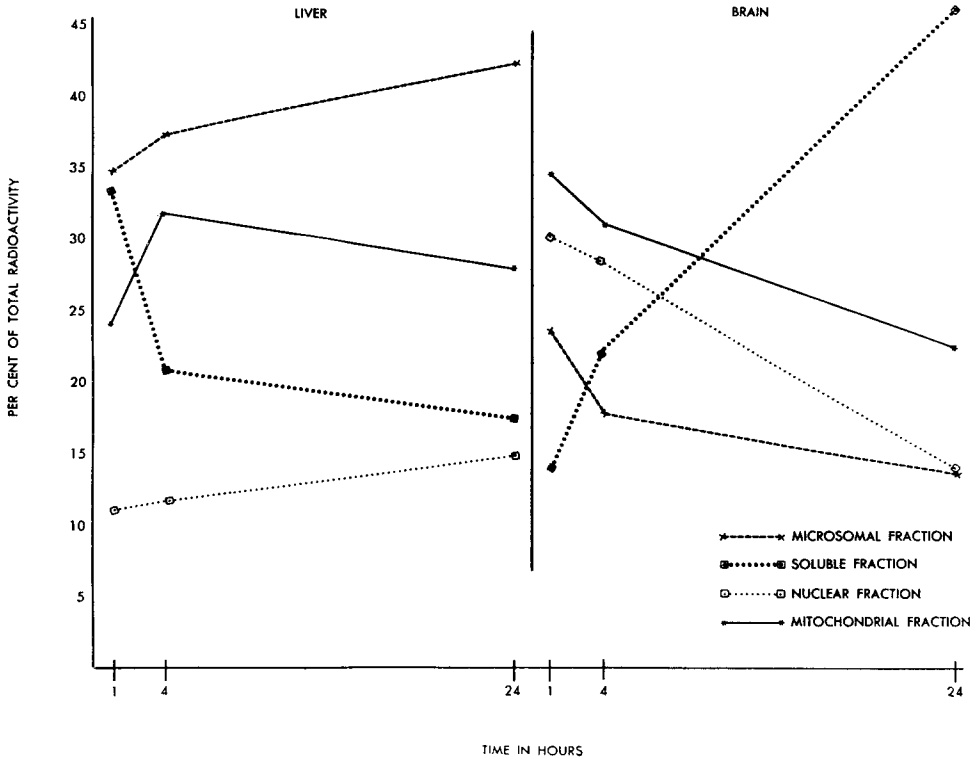

FIG. 2. Distribution of radioactivity in subcellular fractions of brain and liver at various times after administration of ^{35}S-labeled perphenazine to the rat. Each point represents the average from three samples.

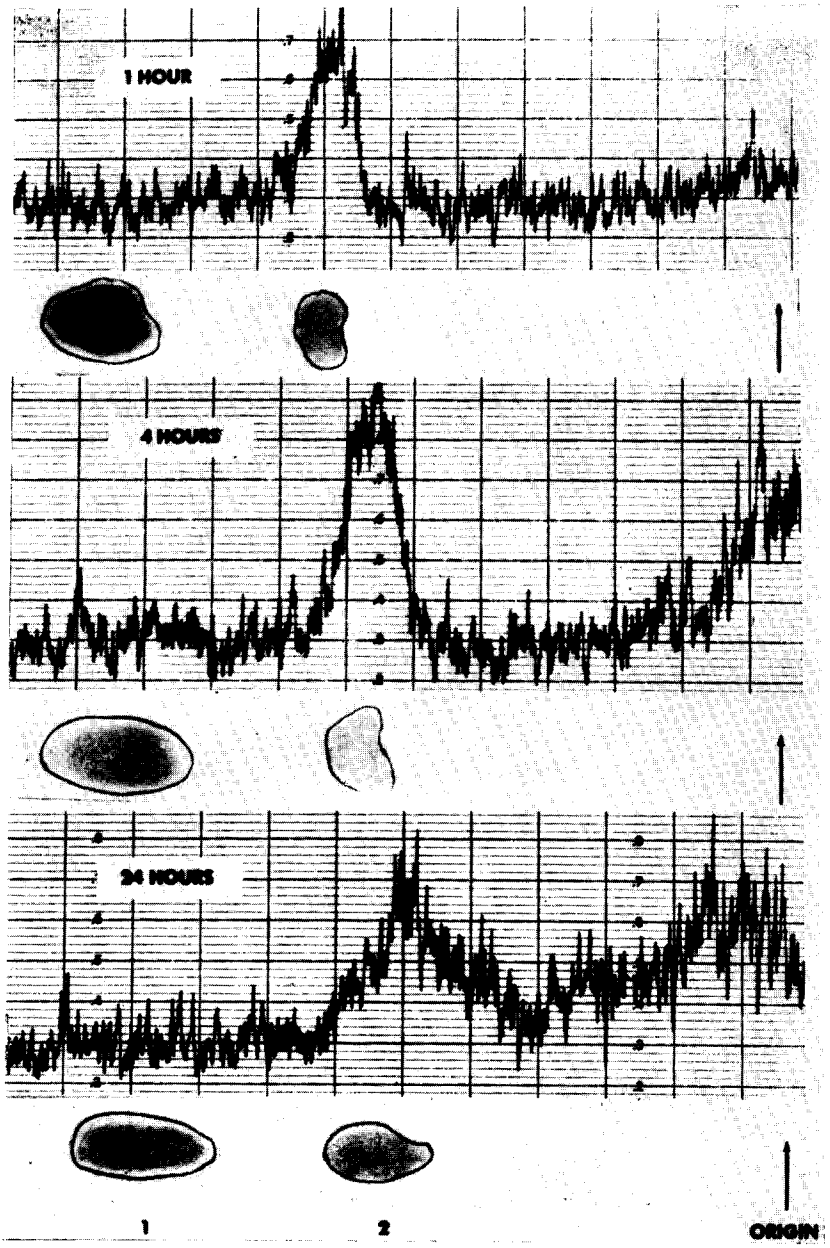

FIG. 1. Chromatographic pattern of radioactivity from urine collected at various times after administration of ^{35}S-labeled perphenazine. On the corresponding paper strips, the position of perphenazine (1) and perphenazine sulfoxide (2) was made visible after spraying with Dragendorff's reagent.

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predominant radioactive spot corresponding to perphenazine sulfoxide carrier. Low levels of radioactivity on paper strips precluded the quantitative evaluation of results (high lipid concentration in the methanolic extracts greatly limited the volume of sample that could be spotted on the paper).

The difference between the liver and brain tissues in extractability and chemical form of the radioactive material may indicate different metabolic fates of perphenazine in these tissues. This seems to be further substantiated by analysis of subcellular distribution of radioactivity, as illustrated in Fig. 2. The results show a clear difference in the distribution pattern at 1 hr; of interest, however, are the marked, but opposite, changes occurring in liver and brain at later time intervals.

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Demethylation of imipramine in male and female rats

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IMIPRAMINE is structurally related to the phenothiazine derivatives currently in wide use as tranquilizing agents; it has an ethylene linkage substituted for the sulfur bridge. Imipramine, however, produces primarily antidepressant effects in patients with depression, although only sedative or tranquilizing actions are found in nondepressed patients.[1] In addition, the antidepressant actions are manifest after a variable period of medication, sometimes a matter of 1 or 2 weeks. Recently Gillette *et al.*[2] and Sulser *et al.*[3] have suggested that both the delayed onset of action of the drug and its antidepressant properties may be ascribed to the formation of the demethylated derivative, desmethylimipramine. This report confirms their observations on the metabolism of the drug in the rat and demonstrates, in addition, a sex difference in the rate of conversion of imipramine to desmethylimipramine.

Imipramine (40 mg/kg) was administered intraperitoneally to male and female rats of the Wistar strain. At various times after injection the brains were removed, frozen on solid carbon dioxide, and subsequently analyzed for their content of imipramine and desmethylimipramine by the method described by Gillette *et al.*[2] for the isolation of desmethylimipramine from rat brain. The compounds were extracted from alkaline brain homogenates with heptane containing 1·5% isoamyl alcohol, the heptane washed with 0·1 N NaOH and the desmethylimipramine and imipramine extracted back into an aqueous phase with phosphate buffer pH 5·9 and 0·1 N HCl, respectively. The fluorescence (activation 295 mμ, fluorescence 415 mμ) of the final extracts in 0·39 N NaOH provided a measure of the amount of each amine. As a routine measure to improve the separation of the two phases, the extracts were frozen after each extraction prior to centrifugation. Standards were included in each set of determinations.