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THE DISTRIBUTION AND EXCRETION OF RADIOACTIVITY AFTER ADMINISTRATION OF ^{35}S-LABELED PERPHENAZINE (Trilafon)*

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Abstract—^{35}S-perphenazine of high specific activity was synthesized, and the identity and radiochemical purity of the product were established. Tissue distribution and excretion of radioactivity were studied in rats after subcutaneous injection of 0·3 mg of ^{35}S-labeled perphenazine dihydrochloride/kg body weight. Maximal concentrations of radioactivity were found in most tissues after 1 hr. Tissues showing the highest peak concentrations were lung, adrenal, liver, kidney, spleen, and pituitary, while the brain parts had somewhat lower, but appreciable, amounts. Blood levels were consistently low. High levels of radioactivity were found in the pituitary gland after 48 hr and remained relatively high even after 6 days. Over 80 per cent of the drug was excreted during a period of 24 hr; most of this (80 per cent) was excreted in the feces, while only 20 per cent could be found in the urine. Experiments performed with female rats in day 19 of pregnancy showed small amounts of radioactivity crossing the placenta. After 24 hr, however, the concentration of radioactivity in the fetus and amniotic fluid was extremely low.

THE widespread use of increasingly potent phenothiazine tranquilizers has prompted us to examine in detail the metabolic disposition in animals of one of the most active member of this class of compounds—namely perphenazine. It was hoped that, by means of a careful study employing the radioisotopically labeled drug, valuable information could be gained that would lead to a better understanding concerning the metabolism, mode of action, and various clinical uses of perphenazine. This report will concern itself with the pattern of distribution of total radioactivity from labeled perphenazine in body tissues, the relationship of this pattern to time after administration of the drug, and the mode and extent of excretion. A subsequent report will deal with the chemical form in which this radioactivity is found in certain tissues and in urine.

MATERIALS AND METHODS

Synthesis of ^{35}S-*labeled perphenazine*

The radiosynthesis was carried out according to the method of Sherlock *et al.*[1] with some modifications necessary for synthesis on a semimicro scale. Fusion of a mixture containing radioactive sulfur, *m*-chlorodiphenylamine, and a small amount of iodine produced radioactive 2-chlorophenothiazine (plus a small amount of the 4-isomer

* Trilafon is the registered trademark of Schering Corporation.

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which was removed by crystallization from toluene). Treatment of a solution of this product in liquid ammonia with sodium amide, followed by the addition of 1-chloro-3-bromopropane, yielded an oily material which, when heated with 1-(2-hydroxyethyl)-piperazine, formed ^{35}S-labeled perphenazine. After vacuum distillation of the free base and repeated recrystallization of the dihydrochloride salt, ^{35}S-labeled perphenazine dihydrochloride was obtained with an over-all yield of approximately 25 per cent, having an initial specific activity of 28 μc/mg.

The synthesized radioactive perphenazine was checked for radiochemical purity and chemical identity. Analysis was carried out by ascending paper chromatography in a *tert*-amyl alcohol:5% acetic acid system. Precise scanning of the chromatograms showed that the preparation was free from radioactive impurities. The single peak coincided with the position of added perphenazine carrier, which was made visible after spraying the paper strip with Dragendorff's reagent.[2] The infrared spectrum of the radioactive material was identical with that of authentic perphenazine. Determination of specific activity with a large excess of added nonradioactive material showed no change in specific activity after repeated crystallization. Determination of pharmacological activity was made by analysis of conditioned avoidance behavior,[3] and no difference in potency was found between the radioactive and authentic compounds.

Determination of ^{35}S radioactivity

Samples of 1 g or less of tissue, feces, or body fluid, as well as aliquots of solutions containing ^{35}S perphenazine with an excess of carrier, were oxidized to inorganic sulfate by a wet digestion procedure that employs a mixture of nitric and perchloric acids containing cupric nitrate. The radioactive sulfate was later precipitated with barium chloride solution. The precipitate was washed, dried, and counted in stainless steel cupped planchets with a Nuclear-Chicago thin-window, gas-flow counter. Each sample (and background) was counted for a minimal number of 4000 total counts. All counts were corrected for background, self-absorption and decay. The corrections for self-absorption were determined from an experimentally constructed curve for ^{35}S-barium sulfate.

Animal studies

Young male rats, weighing approximately 100 g, obtained from the Charles River Breeding Laboratory, were injected subcutaneously with 0·3 mg/kg body weight of ^{35}S-labeled perphenazine dihydrochloride with a specific activity of about 23×10^6 cpm/mg. At appropriate times after injection, groups usually consisting of three animals (the experiments were repeated in many instances for greater reliability of results) were subjected to the following studies. Blood samples were taken by intracardiac puncture from ether-anesthetized rats, which were then immediately sacrificed by decapitation. The brain samples consisted of the entire cerebellum, portions of the cerebral cortex which included both grey and white matter, the hypothalamus, and the medulla (with minimal amount of underlying tissue). The entire pituitary gland was removed from the sella turcica in one piece. The entire liver, the left lobe of the lung, the left kidney, the spleen, the thyroid gland, the adrenals, and muscle samples consisting of gastrocnemius and the lateral muscles of the thigh were also analyzed. Immediately after removal these tissues were weighed, subjected to oxidative digestion,

and analyzed for ^{35}S content. The entire liver was homogenized, and an aliquot of 1 ml was taken for analysis.

Samples of feces and urine for radioanalysis were obtained in the following way. Each rat was placed in an individual metabolism cage before injection; feces were collected free from urine, by employing a method[4] that prevented ingestion of feces. Total feces and an aliquot of urine collected in 24 hr from each animal was taken for analysis.

In a separate experiment three female rats in day 19 of pregnancy were placed in individual cages. The rats were injected subcutaneously with 0·3 mg/kg body weight of ^{35}S-labeled perphenazine dihydrochloride with a specific activity of about $2{\cdot}5 \times 10^6$ cpm/mg and exactly 2 hr later the following operation was performed. An abdominal incision was made in the skin, which was separated from the underlying muscles. A small incision was made in the abdominal wall and the right uterine horn carefully lifted out of the abdominal cavity. A silk suture was placed above the cervix, and the right ovary was left intact. The uterine vessels were brought together and tied with a single suture. The uterus containing the fetuses was then removed without excessive bleeding. After removal of the uterine horn, the abdominal muscles were closed with interrupted sutures and the skin closed with metal clips. The uterine horn was opened, and three fetuses were removed in the following manner: (1) amniotic fluid was first obtained by puncturing the amniotic sac, and the fluid surrounding the three fetuses was collected and combined; (2) each fetus was then removed from its amniotic sac and weighed after making sure that all membranes adhering to the fetus were removed; (3) the placenta was lifted from its maternal connection and the umbilical cord removed, weighed, and placed in a flask. All the rats rapidly recovered from the anesthesia and were kept in separate cages, undisturbed for the next 22 hr. On the following day they were examined and found to be in good condition; 24 hr after injection of the drug the animals were placed under ether anesthesia and the left uterine horn was removed as described, and the same technique was used to obtain amniotic fluid, fetuses, and placenta. After removal of the uterus blood for analysis was obtained from the abdominal aorta. The rat was kept under anesthesia until dead, at which time the entire liver, the right kidney, and a sample of skeletal muscle were removed for analysis.

RESULTS AND DISCUSSION

Tissue distribution of radioactivity from ^{35}S-perphenazine

The concentrations of radioactivity in a number of tissues at various times after drug administration are shown in Table 1. The results indicate a peak concentration of the labeled material in most tissues about 1 hr after administration of the drug. The peak activity is highest in the lung and is followed by the adrenal, liver, and kidney tissue. An appreciable concentration is found in the pituitary and parts of the brain. The lowest values are associated with muscle and blood. The fact that the initial peak activity in the pituitary and in parts of the brain is similar in respect to time to that of the liver indicates that the drug enters the brain probably unchanged in structure.[5] The general pattern of distribution is similar to that found by Fyodorov[6] after subcutaneous injection of chlorpromazine; however, in most tissues, maximal concentrations of radioactivity seem to be reached more rapidly with perphenazine than with chlorpromazine. Of special interest is the extremely high concentration of

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN RAT TISSUE AT VARIOUS TIME INTERVALS AFTER ADMINISTRATION OF ^{35}S-LABELED PERPHENAZINE*

Time	Blood	Cere-bellum	Cere-brum	Hypo-thalamus	Medulla	Pitui-tary	Kidney	Liver	Spleen	Skel. muscle	Adrenal	Thyroid	Lung
6–7 min	135† ±5 (2)	488 ±66 (2)	485 ±93 (2)	473 ±15 (2)	416 ±147 (2)	1539 ±419 (2)	1305 ±336 (2)	781 ±14 (2)	512 ±61 (2)	160 ±18 (2)			
15–17 min	946 ±99 (2)	5725 ±955 (2)	6265 ±35 (2)	5360 ±60 (2)	6285 ±575 (2)	7162 ±1022 (2)	13585 ±965 (2)	12240 ±720 (2)	6615 ±1715 (2)	2380 ±265 (2)			
0·5 hr	722 ±136 (6)	10076 ±800 (3)	11030 ±881 (3)	9054 ±528 (3))	10109 ±566 (3)	14268 ±2037 (3)	20569 ±1126 (3)	19684 ±2416 (3)	14170 ±1836 (3)	3063 ±188 (3)	15740 ±3122 (3)	5828 ±912 (2)	
1 hr	693 ±36 (9)	8490 ±1135 (3)	12000 ±1665 (3)	10891 ±2524 (6)	10082 ±1303 (3)	17262 ±1345 (3)	16663 ±1668 (3)	23254 ±2096 (3))	17865 ±2773 (3)	2829 ±420 (3)	25957 ±3134 (3)	8148 ±1177 (3)	
2 hr	792 ±78 (9)	6967 ±152 (2)	9498 ±146 (3)	7963 ±450 (6)	7647 ±284 (3)	11303 ±1873 (3)	12353 ±86 (3)	24218 ±616 (3)	16012 ±655 (3)	2588 ±268 (3)	23874 ±2026 (3)	7098 ±318 (3)	36727 ±1801 (3)
4 hr	402 ±28 (6)	2325 ±190 (3)	3422 ±334 (2)	2872 ±199 (3)	2889 ±189 (3)	7329 ±2513 (3)	6010 ±392 (3)	17517 ±1519 (3)	5783 ±132 (3)	1049 ±93 (3)	10763 ±942 (3)	3174 ±46 (2)	20500 ±1733 (3)
8 hr	282 ±24 (6)	559 ±58 (3)	785 ±125 (3)	726 ±94 (3)	773 ±20 (3)	3707 ±1169 (3)	2887 ±354 (3)	13539 ±1968 (3)	2076 ±140 (3)	329 ±50 (3)	4322 ±353 (3)	2228 ±603 (2)	7447 ±372 (3)
16 hr	138 ±13 (12)	153 ±9 (6)	214 ±18 (6)	432 ±35 (6)	176 ±26 (6)	3444 ±725 (6)	1218 ±158 (6)	5452 ±495 (6)	474 ±43 (6)	141 ±22 (6)	2295 ±205 (3)	1021 ±97 (2)	1305 ±91 (6)
24 hr	83 ±15 (12)	562 ±265 (6)	189 ±27 (6)	1036 ±375 (6)	257 ±58 (6)	6975 ±2216 (6)	903 ±195 (6)	4444 ±572 (6)	611 ±187 (6)	109 ±27 (6)	1351 ±43 (3)	949 ±116 (3)	713 ±112 (6)
48 hr	84 ±25 (13)	1153 ±479 (7)	326 ±78 (7)	3428 ±1092 (7)	890 ±298 (7)	22857 ±7406 (7)	677 ±113 (7)	2766 ±451 (7)	466 ±62 (7)	67 ±11 (7)	892 ±106 (3)	351 ±82 (3)	268 ±28 (7)
6 day	48 ±4 (3)	173 ±32 (3)	118 ±14 (3)	453 ±224 (3)	194 ±58 (3)	4889 ±627 (3)	453 ±69 (3)	839 ±72 (3)	315 ±35 (3)	22 ±6 (2)			

* Results are expressed as cpm/g (ml) of tissue.
† Mean ± s.e. The figures in parentheses represent the number of tissues (from different animals) under consideration.

radioactivity that is found in the pituitary gland (and to a lesser degree in some parts of the brain) after 48 hr; the magnitude of this concentration is in excess of the peak value found in the same tissue after 1 hr, and relatively large amounts of radioactivity persist even after 6 days. The reason for this unexpected finding is not apparent.

Excretion of ^{35}S

During the first 24 hr after injection, approximately 82 per cent of the total injected radioactivity appeared in the feces and urine (Table 2). Of this 82 per cent, about one-

TABLE 2. EXCRETION OF RADIOACTIVITY DURING 24-HR PERIOD AFTER INJECTION OF ^{35}S-LABELED PERPHENAZINE

Animal	Injected dose (cpm)	Excretion (% of injected dose)		
		Urine	Feces	Total
A	181,000	17·1	70·0	87·1
B	181,000	14·4	69·3	83·7
C	192,000	17·6	64·7	82·3
D	181,000	14·0	61·4	75·4
E	167,000	17·9	64·1	82·0
Mean ± s.e.		16·2 ± 0·8	65·9 ± 1·6	82·1 ± 1·9

fifth was found in the urine and about four-fifths in the feces. Although this type of excretion pattern is not seen with labeled promazine,[7] chlorpromazine,[6] or mepazine,[8] Fyodorov[6] has shown that the radioactivity from labeled chlormepazine is excreted largely in the feces. These results also show that the rate of excretion of perphenazine (or its metabolites) by the rat seems to be faster than that of some related drugs.[6]

Distribution of radioactivity in pregnant rats

The concentration of radioactivity in the fetus, as well as in the placenta and amniotic fluid, at 2 and 24 hr after administration are shown in Table 3. For the sake of

TABLE 3. DISTRIBUTION OF RADIOACTIVITY IN TISSUES OF THE PREGNANT RAT*

	Fetus	Placenta	Amniotic fluid	Blood	Liver	Kidney	Skel. muscle
2 hr	265·0 ± 6·0 (9)†	675·0 ± 33·0 (9)	9·1 ± 1·9 (3)				
24 hr	13·3 ± 0·7 (9)	58·7 ± 5·0 (9)	6·0 ± 0·6 (3)	7·8 ± 1·6 (3)	479·0 ± 55·0 (3)	139·0 ± 27·0 (3)	8·4 ± 2·0 (3)

* Activity is expressed as cpm/g (ml) of tissue.

† Mean ± s.e. The figures in parenthesis represent the number of samples analyzed.

comparison, concentrations at 24 hr of other selected tissues from the same animals are also given. It should be noted, however, that for direct comparison between the results presented in Tables 3 and 1, the difference in specific activity of the injected drug (approximately 10 times) must be taken into consideration. It is apparent from these results that perphenazine (or sulfur-containing metabolites) does cross the placental barrier. The concentration in the fetus is relatively low, however, even at

2 hr; by 24 hr a decrease to about 1/20 of the 2-hr value has occurred. The concentration of radioactivity in the amniotic fluid is extremely small in both of these intervals.

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