Metabolism of Minoxidil, a New Hypotensive Agent I: Absorption, Distribution, and Excretion following Administration to Rats, Dogs, and Monkeys

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Abstract □ Minoxidil (2,4-diamino-6-piperidinopyrimidine 3-oxide), a potent new hypotensive agent, was ^{14}C-labeled in the 2-position of the pyrimidine ring in 17% yield from $Ba^{14}CO_3$. This material was used to study the absorption, distribution, and excretion of the drug in rats, dogs, and monkeys. Following oral administration of a single dose, the drug was rapidly and well absorbed and rapidly eliminated by each species as judged by plasma levels and urinary excretion of unchanged drug and total drug-related materials. Chronic oral administration of the drug at a high level (10 mg/kg) for 30 days slightly increased the rate of clearance of minoxidil and minoxidil-related material from circulation. Water diuresis, resulting from water loading of dogs, caused an even greater increase in the rates of disappearance of the drug and drug-related material. Whole-body autoradiography studies in rats showed that minoxidil was rapidly distributed following its oral and intravenous administration. It was subsequently concentrated, primarily in the excretory system. Minoxidil-related material was detected in aorta walls, but not in the CNS, following both routes of drug administration.

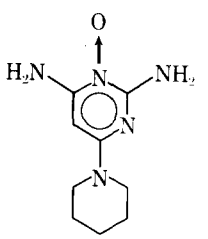
Keyphrases □ Minoxidil, ^{14}C-labeled—absorption, distribution, and excretion after oral and intravenous administration, rats, dogs, and monkeys □ Absorption—^{14}C-labeled minoxidil after oral and intravenous administration, rats, dogs, and monkeys □ Distribution—^{14}C-labeled minoxidil after oral and intravenous administration, rats, dogs, and monkeys □ Excretion—^{14}C-labeled minoxidil after oral and intravenous administration, rats, dogs, and monkeys

Minoxidil (2,4-diamino-6-piperidinopyrimidine 3-oxide, I) is a potent, orally active hypotensive agent. Studies in laboratory animals indicate that its activity is due primarily to a direct relaxant effect on peripheral vascular smooth muscle (1). It has been shown to be orally active in humans (2, 3).

As part of the development of this drug, studies were undertaken in several animal species to determine which most resembles the human in its metabolism of minoxidil. This report is concerned with the preparation of ^{14}C-labeled minoxidil and a study of its disposition, as shown by measurements of unchanged drug and total radioactivity, in three animal species: the rat, dog, and monkey.

EXPERIMENTAL

Preparation of ^{14}C-Labeled Drug—Labeled minoxidil was prepared by a six-step synthetic sequence from barium carbonate-^{14}C in 17% overall yield. Barium carbonate-^{14}C was converted to guanidine-^{14}C hydrochloride *via* barium cyanamide-^{14}C in 84% yield by the method of Bennett (4). Guanidine-^{14}C hydrochloride was then converted to 2,4-diamino-6-hydroxypyrimidine-2-^{14}C monohydrate in 77% yield by the method of VanAllan (5).



I

The ^{14}C-labeled hydroxypyrimidine was heated with phosphorus oxychloride, essentially as described by Israel *et al.* (6) to prepare 2,4-diamino-6-chloropyrimidine-2-^{14}C. By carefully heating the reaction mixture in an oil bath at 95–100° rather than under reflux, a reproducible yield of 69% was obtained in this step. The ^{14}C-labeled chloropyrimidine was oxidized with an equimolar quantity of *m*-chloroperbenzoic acid in ethanol at 3° for 3 hr.

The resulting precipitate of 2,4-diamino-6-chloropyrimidine-2-^{14}C 3-oxide, as the *m*-chlorobenzoic acid salt, was filtered, washed successively with cold ethanol and ether, and air dried. This material, having a specific activity of 1.71 mCi/mmole, was obtained in 68% yield. Its melting point was 199–200° dec. and its IR, UV, and NMR spectra were consistent with the proposed structure.

Anal.—Calc. for $C_{11}H_{10}Cl_2N_4O_3$: C, 41.66; H, 3.18; Cl, 22.36; N, 17.67. Found: C, 41.42; H, 3.41; Cl, 22.25; N, 17.71.

The *m*-chlorobenzoic acid salt of ^{14}C-labeled 2,4-diamino-6-chloropyrimidine 3-oxide (2.72 g) was mixed with 25 ml of piperidine, and the mixture was heated with stirring under nitrogen in an oil bath at 130° to give a clear solution. The solution was refluxed for 1.5 hr, during which time a precipitate formed. After standing overnight at room temperature, the precipitate was filtered. The crude product was washed three times in the filter funnel with 1.5 *N* sodium hydroxide, followed by repeated washing with water. After air drying, 1.10 g (62%) of ^{14}C-minoxidil, 2,4-diamino-6-piperidinopyrimidine-2-^{14}C 3-oxide, having a specific activity of 1.72 mCi/mmole, was obtained. Its melting point was 262–262.5° dec. and its IR, UV, and NMR spectra corresponded to those of authentic minoxidil.

Anal.—Calc. for $C_9H_{15}N_5O$: C, 51.66; H, 7.23; N, 33.46. Found: C, 51.42; H, 7.36; N, 33.42.

When chromatographed in paper and thin-layer systems, the product and its associated radioactivity had mobilities identical to that of authentic minoxidil.

Dosage of Animals and Collection of Samples—Each of three female rats[1], weighing approximately 200 g, was given 1.01 mg (8.2 μCi) of ^{14}C-minoxidil in 1 ml of water by oral intubation. One hour prior to and immediately following administration of the drug, each rat was given 10 ml of deionized water by oral intubation. The

Table I—Urinary Excretion of Minoxidil and Total Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Rat[a]

Hours	Average Percent of Dose Excreted in Urine ± *SD*	
	Total Radioactivity	Minoxidil
0–1	27.8 ± 1.1	15.1 ± 2.4
1–5	38.4 ± 9.9	12.2 ± 7.5
5–11	17.4 ± 11.8	3.0 ± 3.3
11–24	3.49 ± 1.3	0.6 ± 0.6
24–48	0.79 ± 0.74	—
48–72	0.23 ± 0.09	—
Total	88.1 ± 2.6	—

[a] Three animals.

[1] Sprague–Dawley (Upjohn strain).

rats were housed in metabolism cages, designed for the separation and collection of urine and feces, and samples were collected at predetermined intervals for 72 hr. Water and food[2] were permitted *ad libitum*.

In a separate study, each of two 120-g male rats[1], which had been fitted, under ether anesthesia, with bile-duct cannulas [polyethylene (PE-10) tubing], was given 2.55 mg (20.8 μCi) of ^{14}C-minoxidil in 2 ml of water by oral intubation. The rats were placed in restraining cages, and bile was collected continuously for 40 hr in 4-hr fractions. Water was permitted *ad libitum*.

Each of three female dogs[3], weighing approximately 9 kg, was given 5.0 mg (41.0 μCi) of ^{14}C-minoxidil in 10 ml of water by oral intubation. One-half hour before, immediately following, and 1 and 2 hr following administration of the drug, each dog was given 100 ml of deionized water by oral intubation. The dogs were housed in metabolism cages, designed for the separation and collection of urine and feces, and samples were collected at predetermined intervals for 168 hr.

Urine was obtained by catheter up to and including the 16-hr sample and combined with the appropriate voided sample. Blood samples (7 ml) were collected by jugular venipuncture in tubes containing potassium oxalate as an anticoagulant at predetermined times for 168 hr. Except for a 4-hr period following drug administration, water was permitted *ad libitum*. Food[4] was given 12 hr following drug administration and once a day thereafter.

In a separate study, each of four dogs[3], weighing approximately 11 kg, was given a 1.00-mg/kg dose of ^{14}C-minoxidil (8.2 μCi/mg) in 10 ml of water by oral intubation. On each of the next 30 days, each dog was given a 10-mg/kg oral dose of nonradioactive minoxidil in a hard gelatin capsule. This dose was followed on the 31st day with another 1.00-mg/kg dose of ^{14}C-minoxidil. The dogs were kept in metabolism cages during the entire period.

Separate urine collections 0–12 and 12–24 hr following administrations of the labeled drug were made for each dog. Blood samples were collected at predetermined times for 12 hr following labeled drug administrations. Fecal collections were not made.

Each of three male rhesus monkeys, weighing approximately 4 kg, was given a 1.00-mg/kg dose of ^{14}C-minoxidil (8.2 μCi/mg) in 10 ml of water by intubation through a nose tube. The monkeys were placed in restraining chairs, designed for the separate collection of urine and feces, and samples were collected at predetermined intervals for 72 hr.

Blood samples (5 ml) were collected by venipuncture of the femoral vein in the inner midthigh region at predetermined times for 48 hr. The blood was transferred from the syringe to a tube containing potassium oxalate immediately after collection. Water was permitted *ad libitum*, and food[5] was given 8 hr after drug administration and once a day thereafter.

Measurement of Radioactivity in Biological Samples—Blood samples were centrifuged at 1100×*g* for 30 min at 5° to obtain plasma. Feces samples were homogenized with water to a suitable consistency using a blender[6] or homogenizer[7]. Plasma, urine, feces, and bile samples were stored in the frozen state prior to analysis. All samples were counted in liquid scintillation spectrometers[8] interfaced to keypunches[9]. Computations were performed with a computer[9].

Duplicate 0.1- or 0.5-ml aliquots of urine and bile were counted in 15 ml of counting solvent [toluene–dioxane–methanol (350:350:210 v/v) containing 73 g of naphthalene, 4.6 g of 2,5-diphenyloxazole, and 0.080 g of 1,4-bis-2-(5-phenyloxazolyl)benzene/liter]. Counting efficiencies were determined by the internal standard technique using toluene-^{14}C. Duplicate 0.2- or 0.5-ml blood plasma samples and duplicate aliquots, approximately 0.5 g each, of feces homogenates were dried in cellophane sacs at room temperature, combusted[10] (8), and counted. Counting efficiencies were determined by the external standard technique.

[2] Purina rat chow.
[3] Beagle (Upjohn strain).
[4] Purina dog chow.
[5] Purina monkey chow.
[6] Waring Blendor, Waring Products Division, Dynamics Corporation of America, New Hartford, Conn.
[7] Virtis "45," The Virtis Co., Gardiner, N.Y.
[8] Model 3375, Packard Instrument Co., Downers Grove, Ill.
[9] Models 026 and 029 keypunches and model 360/50 computer, International Business Machines, White Plains, N.Y.
[10] With a Peterson-NIH oxidizer.

Table II—Fecal Excretion of Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Rat[a]

Hours	Average Percent of Dose Excreted in Feces ± *SD*
0–24	5.24 ± 1.03
24–48	0.37 ± 0.07
48–72	0.21 ± 0.16
Total	5.8 ± 1.2

[a] Three animals.

Table III—Biliary Secretion of Minoxidil and Total Radioactivity following Oral Administration of ^{14}C-Minoxidil to Bile Duct-Cannulated Rats[a]

	Average Percent of Dose Secreted in Bile	
Hours	Total Radioactivity	Minoxidil
0–4	8.8	0.34
4–8	2.25	0.057
8–12	1.03	—
12–16	0.383	—
16–20	0.182	—
20–24	0.0478	—
24–28	0.0215	—
28–32	0.0167	—
32–36	0.0135	—
36–40	0.0125	—
Total	12.8	—

[a] Two animals.

Preparation of Samples for Chromatography—Samples of urine, blood plasma, and bile containing sufficient radioactivity were applied directly to the paper for chromatography, usually as 2.5-cm streaks. Samples containing lower levels of radioactivity were lyophilized, and the residues were reconstituted at a greater concentration with water or were leached three times with methanol.

The methanol was removed with a nitrogen stream, and the residue was dissolved in a small volume of 1-butanol–methanol (1:2 v/v) for application to paper in a manner similar to that used for whole urine and blood plasma. The leaching procedure resulted in chromatographic metabolite patterns quantitatively identical to those obtained by direct chromatography of whole urine, blood plasma, and bile.

Paper Chromatography and TLC—Paper chromatography was carried out on 86 × 15-cm sheets[11] by a descending technique in the 1-butanol–piperidine–water (82:2:16 v/v) system (System I). Occasionally, the 1-butanol–acetic acid–water (4:1:1 v/v) system (System II) was used. TLC was carried out on 20 × 5-cm films of silica gel, 0.25 mm thick, in the benzene–methanol–ammonium hydroxide (1:1:0.01 v/v) system (System III).

Standard minoxidil was run on each chromatogram. In certain cases, minoxidil was mixed with samples prior to chromatography to act as an internal standard. Following solvent development, the chromatograms were viewed under UV light to locate the standard and, when possible, drug-related materials by their UV absorption.

Quantification of Minoxidil by Radiochromatogram Scanning—Paper and thin-layer chromatograms, when they contained sufficient radioactivity, were scanned for radioactivity using chromatogram scanners[12] having Geiger detectors. The resulting chart-strip tracings were integrated with a mechanical planimeter[13] or with an electronic digitizer system[14] to determine quantitatively the relative distribution of radioactivity along the length of the chromatogram.

When a chromatogram did not contain sufficient radioactivity for quantification by Geiger detection, it was cut into sequential

[11] Whatman No. 2 paper, W. and R. Balston, Ltd., London, England.
[12] Model 880 paper and model 885 thin-layer scanners, Vanguard Instrument Co., North Haven, Conn.
[13] Model 700AR Lasico, Los Angeles Scientific Instruments Co., Los Angeles, Calif.
[14] Model 9864 digitizer and model 9810 calculator, Hewlett-Packard Corp., Loveland, Colo.

Table IV—Plasma Levels and Areas under Plasma Level Curves for Minoxidil and Total Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Monkey at a Level of 1 mg/kg[a]

Hours	Average Plasma Concentration ± *SD*, μg/ml		Cumulative Average Area under Plasma Level Curve ± *SD*, hr × μg/ml	
	Total Radioactivity	Minoxidil	Total Radioactivity	Minoxidil
0.25	0.343 ± 0.144	0.037 ± 0.021	0.0429 ± 0.0181	0.005 ± 0.003
0.5	0.490 ± 0.221	0.037 ± 0.014	0.147 ± 0.062	0.014 ± 0.007
1	0.371 ± 0.100	0.028 ± 0.002	0.340 ± 0.180	0.026 ± 0.011
4	0.123 ± 0.046	0.013 ± 0.005	1.17 ± 0.33	0.102 ± 0.033
8	0.0324 ± 0.0160	0.0028 ± 0.0025	1.48 ± 0.43	0.133 ± 0.048
14	0.0058 ± 0.0029	—	1.58 ± 0.69	—
24	0.0014 ± 0.0010	—	1.62 ± 0.70	—
48	0.0006 ± 0.0002	—	1.64 ± 0.72	—

[a] Three animals.

segments (1 cm in the case of paper chromatograms and 0.5 cm in the case of thin-layer chromatograms). Then the segments, each in a vial with 10 ml of counting solvent containing 3% water, were counted by liquid scintillation spectrometry. The resulting raw data were processed by the computer system to give a plot of the distribution of radioactivity along the length of the chromatogram, in a format similar to that of a chart-strip tracing, together with a tabular printout of derived data. Based on the plot, the tabular data were used to quantify the relative distribution of radioactivity along the length of the chromatogram.

Whole-Body Autoradiography—Each of 10 male rats[1], weighing approximately 110 g, was given 2.25 mg (18.2 μCi) of ^{14}C-minoxidil, five by oral intubation and five by intravenous administration in the tail vein. At intervals of 0.5, 2, 4, 8, and 24 hr following oral administration and 1 and 10 min and 0.5, 2, and 4 hr following intravenous administration, the rats were quickly frozen by immersion in dry ice-cooled acetone while under ether anesthesia. The rats were then embedded by freezing in blocks of 4% aqueous carboxymethylcellulose sodium and sectioned for whole-body autoradiography essentially as described by Ullberg (7).

Sections 30 μm thick were collected on cellophane tape[15] as they were being cut with a microtome[16] equipped with a 55° tungsten carbide-tipped knife[16] at −20° in a cryostat[17]. The sections were allowed to dehydrate in the cryostat and then were placed in contact with X-ray film[18] for 1–6 weeks at −15°. The film was developed by standard methods.

RESULTS

As shown in Tables I and II, the rat eliminated minoxidil rapidly, primarily *via* urine. Following a 5-mg/kg oral dose of ^{14}C-minoxidil, 88% of the radioactivity was excreted in urine and 5.8% was excreted in the feces. More than 80% of the dose was accounted for in urine during the 11 hr immediately following drug administration. Approximately 35% of the drug-related material excreted in urine was minoxidil. Bile duct-cannulated rats (Table III) secreted an average of 12.8% of a 21-mg/kg oral dose of ^{14}C-minoxidil in bile over 40 hr. Most of this amount (11% of the dose) was secreted during the 8 hr following drug administration. Less than 4% of the drug-related material secreted in bile during this 8-hr period was minoxidil.

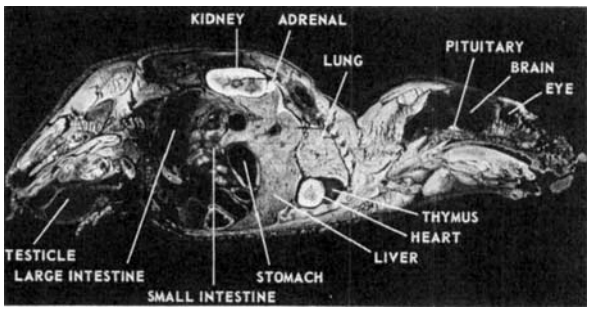


Figure 1—*Distribution of radioactivity (light areas) in a rat 1 min following intravenous administration of ^{14}C-minoxidil. Film was exposed to the tissue slice for 1 week.*

Plasma levels of minoxidil, total drug-related material, and areas under plasma level curves (expressed as microgram-equivalents of drug) following oral administration of 1 mg of minoxidil/kg to monkeys are presented in Table IV. An average peak plasma level of 0.037 μg of minoxidil/ml was attained 0.25–0.5 hr after drug administration. Minoxidil and total radioactivity levels in plasma decreased rapidly thereafter. Unchanged drug accounted for only about 10% of the total drug-related material in circulation.

Minoxidil disappeared from plasma with a 1.9-hr average half-life during the 8-hr interval following attainment of the peak plasma level. Excretion of minoxidil by the monkey also was rapid and primarily *via* urine (Tables V and VI). Approximately 94% of the dose was excreted in urine and 2.68% was excreted in feces. More than 90% of the dose was excreted in urine during the 14 hr immediately following drug administration. Approximately 10% of the drug-related material excreted in urine was minoxidil.

Plasma levels of minoxidil, total drug-related material, and areas under plasma level curves (expressed as microgram-equivalents of drug) following oral administration of 0.55 mg ^{14}C-minoxidil/kg to water-loaded dogs are presented in Table VII. An average peak plasma level of 0.37 μg of minoxidil/ml was observed at the first sampling time, 15 min after drug administration.

Minoxidil disappeared from plasma with a 0.8-hr average half-life during the 5-hr period following the peak plasma level. Excretion of radioactivity by the dog was rapid and primarily *via* urine (Tables VIII and IX). Nearly 91% of the dose was excreted in urine

Table V—Urinary Excretion of Minoxidil and Total Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Monkey[a]

Hours	Average Percent of Dose Excreted in Urine	
	Total Radioactivity	Minoxidil
0–4	66.9 ± 6.8	5.4 ± 1.6
4–8	18.5 ± 6.3	3.6 ± 2.4
8–14	7.1 ± 1.4	0.66 ± 0.47
14–24	1.13 ± 0.23	—
24–48	0.47 ± 0.04	—
48–72	0.055 ± 0.018	—
Total	94.2 ± 0.3	—

[a] Three animals.

Table VI—Fecal Excretion of Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Monkey[a]

Hours	Average Percent of Dose Excreted in Feces ± *SD*
0–24	0.006 ± 0.008
24–72	1.99 ± 0.35
72–96	0.69 ± 0.18
Total	2.68 ± 0.16

[a] Three animals.

[15] Scotch Magic mending tape, No. 810, 3M Co., St. Paul, Minn.
[16] Jung, Model K, William J. Hacker and Co., West Caldwell, N.J.
[17] Harris Manufacturing Co., Cambridge, Mass.
[18] Kodak No-Screen X-ray film, Eastman Kodak Co., Rochester, N.Y.

Table VII—Plasma Levels and Areas under Plasma Level Curves for Minoxidil and Total Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Dog at a Level of 0.55 mg/kg[a]

Hours	Average Plasma Concentration ± *SD*, μg/ml		Cumulative Average Area under Plasma Level Curve ± *SD*, hr × μg/ml	
	Total Radioactivity	Minoxidil	Total Radioactivity	Minoxidil
0.25	0.675 ± 0.093	0.37 ± 0.07	0.084 ± 0.012	0.047 ± 0.008
0.75	0.584 ± 0.053	0.19 ± 0.01	0.399 ± 0.048	0.187 ± 0.024
1.25	0.494 ± 0.036	0.11 ± 0.02	0.67 ± 0.06	0.261 ± 0.017
1.75	0.398 ± 0.038	0.083 ± 0.024	0.89 ± 0.07	0.310 ± 0.011
2.25	0.322 ± 0.038	0.048 ± 0.020	1.07 ± 0.09	0.343 ± 0.018
2.75	0.251 ± 0.035	0.031 ± 0.014	1.21 ± 0.11	0.363 ± 0.026
3.5	0.175 ± 0.025	0.013 ± 0.005	1.37 ± 0.12	0.379 ± 0.032
5	0.098 ± 0.020	0.0053 ± 0.0041	1.58 ± 0.15	0.303 ± 0.039
8	0.0385 ± 0.0048	0.0016 ± 0.0011	1.78 ± 0.18	0.403 ± 0.046
12	0.0174 ± 0.0017	—	1.89 ± 0.19	—
16	0.0104 ± 0.0017	—	1.95 ± 0.19	—
24	0.0050 ± 0.0003	—	2.01 ± 0.19	—
36	0.0021 ± 0.0006	—	2.06 ± 0.19	—
48	0.0012 ± 0.0002	—	2.08 ± 0.19	—
72	0.0006 ± 0.0001	—	2.10 ± 0.19	—
96	0.0004 ± 0.0002	—	2.11 ± 0.19	—
120	0.0006 ± 0.0002	—	2.13 ± 0.19	—
144	0.0005 ± 0.0001	—	2.14 ± 0.19	—
168	0.0004 ± 0.0001	—	2.15 ± 0.19	—

[a] Three animals.

and 7.1% was excreted in feces. More than 80% of the dose was excreted in urine during the 12 hr immediately following drug administration. Approximately 13% of the drug-related material excreted in urine was minoxidil.

Plasma levels of minoxidil, total drug-related material, and areas under plasma level curves (expressed as microgram-equivalents of minoxidil) following oral administration of 1 mg of ^{14}C-minoxidil/kg to dogs before and after 30 successive 10-mg/kg daily oral doses of minoxidil are presented in Table X. Before chronic drug treatment, an average peak plasma level of 0.84 μg of minoxidil/ml was observed at the first sampling time, 15 min after drug administration; the corresponding value after chronic drug treatment was 0.535 μg/ml and occurred about 45 min following administration of the labeled drug.

For approximately 5 hr following attainment of the peak plasma level, minoxidil disappeared with an average half-life of 1.2 hr prior to, and 1.0 hr following, chronic drug treatment. The corresponding areas under the plasma level curves are presented in Table XI. The cumulative areas under the minoxidil-level curves during the 12 hr following drug administration were 1.27 hr × μg/ml prior to, and 1.08 hr × μg/ml following, chronic drug treatment.

Urinary excretion of radioactivity during this chronic study is presented in Table XII. Both before and after chronic drug treatment, approximately 89% of the radioactive dose was excreted in urine during the 24 hr following administration of the labeled drug. In each case, more than 80% of the dose was excreted during the first 12 hr. Approximately 18% of the drug-related material excreted in urine was minoxidil in both cases.

Within 1 min after injecting the rat intravenously with ^{14}C-minoxidil, radioactivity had penetrated into most tissues (Fig. 1). The distribution was relatively uniform except for a notable lack of radioactivity in the brain, testicles, stomach, large intestine, spleen, and bone. The level of radioactivity was low in the thymus. Ten minutes after intravenous injection (Fig. 2), levels of radioactivity were highest in the kidneys, liver, bladder, and aorta walls, although most other tissues, including the testicles and stomach, contained moderate concentrations of radioactivity. Little, if any, radioactivity was present in the brain and large intestine.

One-half hour following intravenous injection (Fig. 3), radioactivity levels were relatively high in the liver, kidneys, small intestine, bladder, and aorta walls. Little radioactivity remained in other tissues, including blood, and none was apparent in the brain. Four hours following intravenous administration, levels of radioactivity were relatively high in the large intestine and bladder, moderate in the liver, low in the kidneys, and not detectable in most other tissues, including the brain and blood.

One-half hour after oral administration of ^{14}C-minoxidil to the rat, the radioactivity (Fig. 4) was most concentrated in the kidneys, stomach, bladder, and small intestine. The liver and aorta walls had nearly as high a concentration. Other tissues had a rather uniform concentration but to a lesser degree. The brain was de-

Table VIII—Urinary Excretion of Minoxidil and Total Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Dog[a]

Hours	Average Percent of Dose Excreted in Urine ± *SD*	
	Total Radioactivity	Minoxidil
0–0.5	11.3 ± 2.7	4.0 ± 0.9
0.5–1	12.6 ± 3.0	3.0 ± 0.8
1–1.5	13.0 ± 1.1	1.9 ± 0.5
1.5–2	8.9 ± 0.9	1.0 ± 0.3
2–2.5	6.7 ± 0.4	0.5 ± 0.2
2.5–3	5.9 ± 0.6	0.4 ± 0.3
3–4	7.4 ± 1.6	0.4 ± 0.3
4–6	8.4 ± 1.3	0.2 ± 0.2
6–8	4.20 ± 0.79	0.03 ± 0.1
8–12	4.50 ± 0.54	0.05 ± 0.1
12–16	2.85 ± 0.95	0.05 ± 0.1
16–24	2.29 ± 0.36	—
24–36	1.10 ± 0.98	—
36–48	0.90 ± 0.73	—
48–72	0.41 ± 0.21	—
72–96	0.103 ± 0.024	—
96–120	0.065 ± 0.026	—
120–144	0.038 ± 0.010	—
144–168	0.042 ± 0.032	—
Total	90.7 ± 2.3	—

[a] Three animals.

Table IX—Fecal Excretion of Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Dog[a]

Hours	Average Percent of Dose Excreted in Feces ± *SD*
0–24	0.016 ± 0.014
24–48	5.92 ± 2.34
48–72	0.89 ± 0.82
72–96	0.127 ± 0.099
96–120	0.068 ± 0.077
120–144	0.044 ± 0.046
144–168	0.019 ± 0.020
Total	7.1 ± 3.1

[a] Three animals.

Table X—Plasma Levels of Minoxidil and Total Radioactivity following Oral Administration of ^{14}C-Minoxidil at a Level of 1 mg/kg to the Dog before and after Chronic Administration of Minoxidil[a]

	Average Plasma Concentration ± *SD*, μg/ml			
	Before		After	
Hours	Total Radioactivity	Minoxidil	Total Radioactivity	Minoxidil
0.25	1.29 ± 0.14	0.84 ± 0.27	0.466 ± 0.227	0.333 ± 0.159
0.75	1.20 ± 0.14	0.51 ± 0.05	1.07 ± 0.21	0.535 ± 0.081
1.25	1.05 ± 0.10	0.35 ± 0.04	1.07 ± 0.13	0.390 ± 0.038
1.75	0.93 ± 0.12	0.23 ± 0.07	0.96 ± 0.10	0.260 ± 0.032
2.25	0.80 ± 0.16	0.18 ± 0.03	0.82 ± 0.07	0.190 ± 0.037
2.75	0.71 ± 0.18	0.14 ± 0.03	0.68 ± 0.05	0.135 ± 0.041
3.5	0.63 ± 0.21	0.10 ± 0.01	0.51 ± 0.057	0.080 ± 0.030
5.0	0.417 ± 0.168	0.039 ± 0.005	0.293 ± 0.052	0.030 ± 0.015
8.0	0.172 ± 0.077	0.007 ± 0.002	0.118 ± 0.029	0.007 ± 0.004
12.0	0.067 ± 0.028	0.001 ± 0.001	0.050 ± 0.009	0.001 ± 0.002

[a]Four animals.

Table XI—Areas under Minoxidil and Total Radioactivity Plasma Level Curves following Oral Administration of ^{14}C-Minoxidil to the Dog before and after Chronic Administration of Minoxidil[a]

	Average Cumulative Area under Plasma Level Curve ± *SD*, hr × μg/ml			
	Before		After	
Hours	Total Radioactivity	Minoxidil	Total Radioactivity	Minoxidil
0.25	0.161 ± 0.055	0.105 ± 0.034	0.058 ± 0.028	0.042 ± 0.020
0.75	0.784 ± 0.195	0.444 ± 0.113	0.442 ± 0.137	0.258 ± 0.079
1.25	1.35 ± 0.25	0.662 ± 0.122	0.98 ± 0.22	0.489 ± 0.102
1.75	1.84 ± 0.30	0.809 ± 0.133	1.48 ± 0.27	0.652 ± 0.106
2.25	2.27 ± 0.35	0.911 ± 0.148	1.93 ± 0.30	0.764 ± 0.103
2.75	2.65 ± 0.43	0.092 ± 0.160	2.30 ± 0.30	0.846 ± 0.098
3.5	3.15 ± 0.57	1.08 ± 0.163	2.75 ± 0.31	0.926 ± 0.089
5.0	3.94 ± 0.84	1.19 ± 0.165	3.36 ± 0.30	1.01 ± 0.088
8.0	4.82 ± 1.20	1.26 ± 0.166	3.97 ± 0.30	1.06 ± 0.097
12.0	5.30 ± 1.41	1.27 ± 0.161	4.31 ± 0.33	1.08 ± 0.104

[a]Four animals.

void of radioactivity. The relative distribution of radioactivity 2 hr following oral drug administration (Fig. 5) was similar to that at 30 min. However, the levels of radioactivity in the stomach and kidneys had decreased somewhat.

Four hours following oral drug administration (Fig. 6), radioactivity levels were relatively high in the bladder, the large intestine, and some sections of the small intestine; moderate in the stomach, liver, and kidneys; very low in most other tissues; and lacking in the brain. Eight hours following oral administration, the large intestine had the highest levels of radioactivity relative to other tissues. The liver and kidneys, as well as the compact portion of bone, had somewhat lower levels. Most other tissues contained very little radioactivity; none was detectable in the brain.

Twenty-four hours following oral drug administration (Fig. 7), the large intestine contained the highest level of radioactivity. Somewhat lower levels were observed in the stomach and in the compact portion of bone. The liver and kidneys had very low levels; other tissues, including the brain, appeared to be devoid of radioactivity.

DISCUSSION

As judged by plasma levels and excretion of minoxidil and by total radioactivity, labeled minoxidil, administered as a single oral dose, was well absorbed and rapidly cleared from the circulation and the body by the rat, dog, and monkey. Each species absorbed nearly 90% or more of the drug based on the amount of radioactivity excreted in urine. More than 80% of the administered drug was cleared from the body of each species within 12 hr. With the monkey and dog, peak plasma levels of minoxidil were attained 15–45 min following drug administration. The disappearance of minoxidil from plasma was rapid, corresponding to average half-lives of 1.9 and 1.2 hr for the monkey and dog, respectively. This difference in half-lives is significant ($p < 0.10$ by t test).

Minoxidil was at least as well absorbed by the human (9), with 97% of the radioactivity associated with an orally administered dose of labeled drug appearing in urine. It was cleared somewhat more slowly from circulation by the human, with a plasma half-life

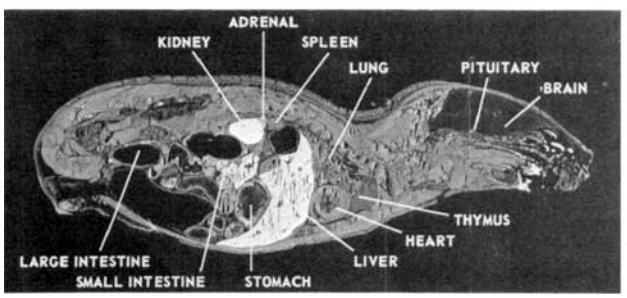


Figure 2—*Distribution of radioactivity (light areas) in a rat 10 min following intravenous administration of ^{14}C-minoxidil. Film was exposed to the tissue slice for 1 week.*

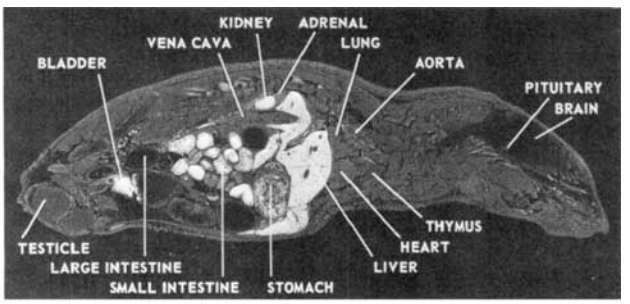


Figure 3—*Distribution of radioactivity (light areas) in a rat 30 min following intravenous administration of ^{14}C-minoxidil. Film was exposed to the tissue slice for 1 week.*

Table XII—Urinary Excretion of Minoxidil and Total Radioactivity following Oral Administration of ^{14}C-Minoxidil to the Dog before and after Chronic Administration of Minoxidil[a]

	Average Percent of Dose Excreted in Urine ± *SD*			
	Before		After	
Hours	Total Radioactivity	Minoxidil	Total Radioactivity	Minoxidil
0–12	81.0 ± 2.9	17.1 ± 5.1	81.3 ± 4.5	18.5 ± 2.2
12–24	8.17 ± 1.90	0.2 ± 0.2	7.34 ± 2.39	0.3 ± 0.1
Total	89.2 ± 3.0	17.3 ± 5.3	88.7 ± 3.2	18.8 ± 2.2

[a] Four animals.

of 4.2 hr, and more slowly from the body, with 70% of the dose appearing in urine within 12 hr after drug administration.

Oral administration of 30 successive daily doses of minoxidil, at a level of 10 mg/kg, to dogs resulted in an apparent small increase in the rate of removal of minoxidil from circulation. When 1.00-mg/kg doses of labeled minoxidil were administered, the average half-life for removal of minoxidil from plasma and the area under the plasma minoxidil level *versus* time curve both were 20% lower following, as compared to immediately preceding, chronic drug treatment. The average half-life changed from 1.2 to 1.0 hr, and the area changed from 1.27 to 1.08 hr × μg/ml. These small changes ($p < 0.05$ for half-life and $p < 0.20$ for area differences by paired t tests) could have resulted from an increase in the rate of drug metabolism, caused by stimulation of an enzyme system metabolizing minoxidil, or from an increase in urinary excretion rates for the drug and its metabolites.

The dogs used in the single-dose kinetic study, in which a 0.55-mg/kg dose of labeled minoxidil was used, were water loaded to obtain frequent urine samples. The dogs used in the chronic study, however, were not water loaded since frequent urine sampling was not planned. The water diuresis resulting from water loading appears to have increased the rate of removal of minoxidil-related material from circulation. The half-life for removal of minoxidil from plasma and the area under the plasma minoxidil level curve (0–8 hr) for the water-loaded dogs both were approximately 35% lower than the corresponding values obtained following the first dose of radioactive drug in the chronic study ($p < 0.10$ for half-life and area differences by paired t tests). This effect is even greater than that due to chronic drug administration. This effect could be due to an increase in urinary excretion rates for the drug and its metabolites caused by their decreased renal reabsorption under conditions of water diuresis.

Biliary secretion of minoxidil-related materials following oral administration of the drug to the rat was significant, accounting for 13% of the dose. Since only 6% of an oral dose (in a separate study) was accounted for in feces of normal rats, there is some indication of enterohepatic recirculation of the drug and its metabolites by the rat.

As determined by autoradiography, radioactivity associated with labeled minoxidil was very rapidly and widely distributed in the body of the rat following intravenous and oral drug administration. Shortly thereafter, radioactivity began concentrating, primarily in the digestive and excretory system. These systems, particularly the liver, kidneys, bladder, stomach, and intestines, generally contained the highest relative concentrations of radioactivity at increasing times following drug administration. These concentrations were relative to other tissues of the body, since previous excretion studies showed that at least 38, 41, 57, 76, and 92% of the administered radioactivity had been eliminated by the rat 1, 2, 4, 8, and 24 hr, respectively, following oral administration of the labeled drug.

Although accumulation of a drug (and, particularly, only the radioactivity initially associated with the drug) in a certain tissue does not necessarily indicate the site of pharmacological action, certain observations are informative. Little, if any, minoxidil-related material was detected in the central nervous system relative to other tissues at any time following drug administration. Radioactivity concentrated in aorta walls, but not in nonsmooth vascular

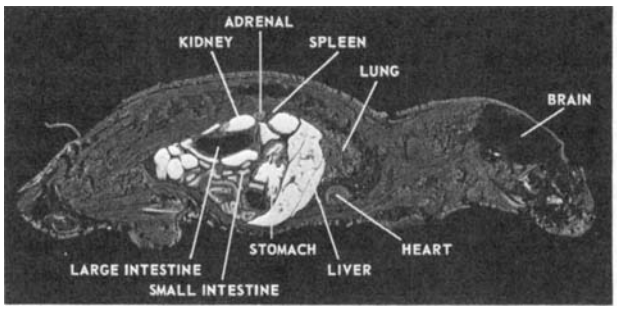


Figure 4—*Distribution of radioactivity (light areas) in a rat 30 min following oral administration of ^{14}C-minoxidil. Film was exposed to the tissue slice for 1 week.*

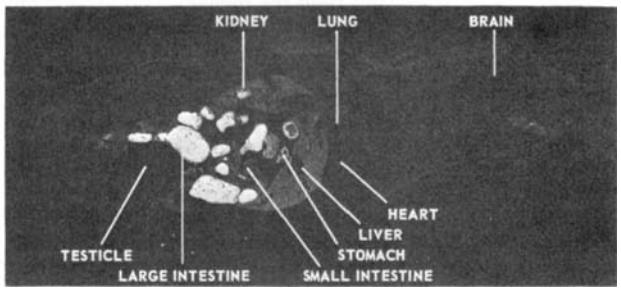


Figure 6—*Distribution of radioactivity (light areas) in a rat 4 hr following oral administration of ^{14}C-minoxidil. Film was exposed to the tissue slice for 2 weeks.*

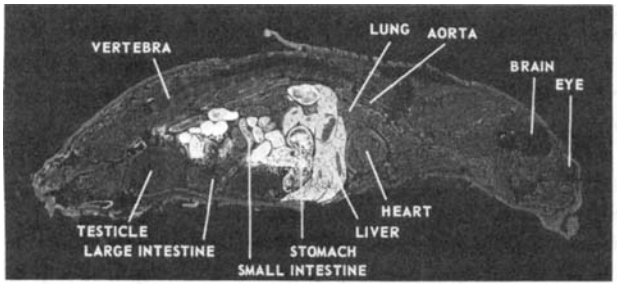


Figure 5—*Distribution of radioactivity (light areas) in a rat 2 hr following oral administration of ^{14}C-minoxidil. Film was exposed to the tissue slice for 2 weeks.*

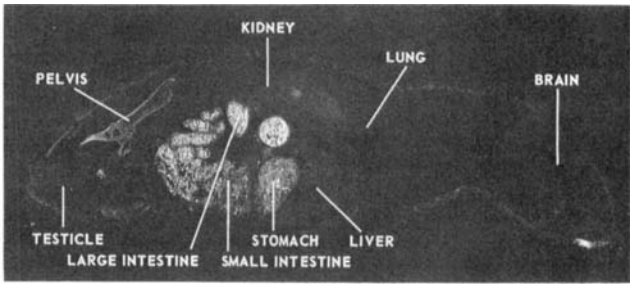


Figure 7—*Distribution of radioactivity (light areas) in a rat 24 hr following oral administration of ^{14}C-minoxidil. Film was exposed to the tissue slice for 6 weeks.*

muscle such as the myocardium, for at least 2 hr following drug administration. This concentration may well have continued beyond 2 hr, as reported by Pluss *et al.* (10), without being detected because of the relatively low sensitivity of whole-body section autoradiography. In any event, these observations are consistent with minoxidil having a direct relaxant effect on peripheral vascular smooth muscle.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 17, 1974, from the *Research Laboratories, The Upjohn Company, Kalamazoo, MI 49001*

Accepted for publication January 21, 1975.

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Metabolism of Minoxidil, a New Hypotensive Agent II: Biotransformation following Oral Administration to Rats, Dogs, and Monkeys

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Abstract □ The biotransformation of minoxidil (2,4-diamino-6-piperidinopyrimidine 3-oxide) was studied in the rat, dog, and monkey and compared to reported results in the human. Chromatographic profiles of urinary metabolites show that each species excreted substantially the same metabolites but in quite different relative amounts. The monkey and the human exhibited similar metabolite profiles, whereas the dog and rat were quantitatively different from each other and from the monkey and human. The major excretory product for the monkey and human was a glucuronide conjugate of minoxidil. Substantially smaller amounts of unchanged minoxidil, 2,4-diamino-6-(4′-hydroxypiperidino)pyrimidine 3-oxide, and more polar metabolites also were excreted by these two species. The major excretory product in the rat was unchanged minoxidil. Almost as much (combined) of the two acidic metabolites, 2,4-diamino-6-(4′-carboxy-*n*-butylamino)pyrimidine and its 3-oxide, also were produced. Smaller amounts of the glucuronide of minoxidil, 2,4-diamino-6-(4′-hydroxypiperidino)pyrimidine 3-oxide, its 3′-hydroxy isomer, and 2,4-diamino-6-piperidinopyrimidine also were excreted by the rat. The major metabolite of minoxidil excreted by the dog was the 4′-hydroxy metabolite. Smaller amounts of unchanged minoxidil and polar metabolites and much smaller amounts of the glucuronide of minoxidil, the 3′-hydroxy metabolite, and 2,4-diamino-6-piperidinopyrimidine also were excreted by the dog. Evidence was obtained for a glucuronide conjugate of the 4′-hydroxy metabolite in this species. The major circulatory material in dog plasma was the 4′-hydroxy metabolite, whereas it was the glucuronide of minoxidil in monkey plasma.

Keyphrases □ Minoxidil—biotransformation in rat, dog, and monkey after oral administration, compared to reported human metabolism □ Metabolism, minoxidil—biotransformation in rat, dog, and monkey after oral administration, compared to reported human metabolism

As part of a program to develop minoxidil (2,4-diamino-6-piperidinopyrimidine 3-oxide, I) as an orally active hypotensive agent, metabolism studies with the drug were undertaken in three animal species: the rat, dog, and monkey. Previous reports described studies that showed that minoxidil, following its oral administration, was rapidly and well absorbed, rapidly and widely distributed, and rapidly eliminated, primarily *via* urine, by the rat, dog, and monkey (1) and by the human (2). Tissue distribution studies in the rat using ^{14}C-minoxidil were reported previously (1, 3).

These results, based on measurements of unchanged drug and total drug-related material, were not sufficient for selecting one species as being most like the human in its disposition of minoxidil. Such a selection was of particular importance for this drug because it causes an apparently species-specific, right-atrial lesion in the dog (4). Therefore, studies were undertaken to compare the biotransformation of minoxidil in the three animal species and to see if at least one of them was similar to the human in this aspect of the drug's disposition. These studies were extended to identify the metabolites of minoxidil produced by each animal species and to determine the kinetics of absorption, distribution, and excretion of the unchanged drug and its metabolites by each species.

This report is concerned with comparing the biotransformation of minoxidil in the three animal species and humans, including characterization, isolation, identification, and quantification of metabolites in urine and blood plasma. A subsequent report will deal with the kinetics of absorption, distribution, and excretion of minoxidil and its metabolites by each animal species.