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# Disposition of Hydralazine in Man and a Specific Method for Its Determination in Biological Fluids


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**Abstract** □ A sensitive analytical procedure was developed for the measurement of the concentrations of hydralazine in human plasma and urine. The method is based on the formation of the hydrazone derivative of hydralazine with *p*-methoxybenzaldehyde. Specificity of the procedure for unchanged drug was demonstrated by inverse isotope dilution analysis following administration of hydralazine-$^{14}$C to humans. Plasma levels and urinary excretion of hydralazine were measured in healthy subjects after single oral therapeutic doses.



**Keyphrases** □ Hydralazine-$^{14}$C—plasma levels and urinary excretion in man, spectrophotometric and inverse isotope dilution analysis in biological fluids □ Plasma levels—hydralazine-$^{14}$C after oral administration, man □ Urinary excretion—hydralazine-$^{14}$C after oral administration, man □ Spectrophotometry—analysis, hydralazine in plasma and urine □ Inverse isotope dilution—analysis, hydralazine in plasma and urine


Hydralazine[1] (1-hydrazinophthalazine), a potent, peripherally acting vasodilator with a prompt and general blood pressure-lowering effect, has been used for the treatment of essential hypertension for many years. Although many pharmacological and clinical papers have appeared, relatively little is known concerning its biological disposition.

The fate of hydralazine in the rat was investigated (1) with the aid of $^{14}$C-labeled material. Plasma levels of hydralazine in man after oral administration were reported (2, 3), and average peak levels of 0.23 μg/ml were attained after a daily dose of 600 mg. In another study (4), 150 mg of hydralazine was given four times daily for an extended period to one hypertensive subject and peak plasma levels reached a plateau of 1.5 μg/ml. Recently, plasma levels 2 hr postadministration obtained in hypertensive patients after 5–20 weeks of hydralazine treatment were reported (5).

The objective of the present study was to obtain information on the biological fate of hydralazine in healthy human subjects after administration of single doses in the clinically effective dose range. Based on the reported data, it was anticipated that plasma levels after administration of a single 100-mg dose would decline rapidly. Therefore, the sensitivity and specificity of existing assay methods had to be examined carefully.

The method published by Perry *et al.* (3) utilized

[1] Apresoline, Ciba-Geigy Corp.

the reaction of hydralazine with ninhydrin. Schulert (4) found that the ninhydrin method gave incomplete recovery and variable results, so he developed an alternative method based on the condensation of hydralazine with *p*-hydroxybenzaldehyde. Unfortunately, other investigators (5) encountered difficulties in their attempts to repeat Schulert's procedure and had to introduce modifications to obtain reproducible results.

Both methods were tested in this laboratory, but neither the original nor the modified method of Schulert afforded the desired reproducibility with the necessary accuracy, particularly at low plasma concentrations. Furthermore, the earlier investigators did not provide evidence that the reported methods were specific for unchanged hydralazine in the presence of its metabolites. Therefore, it became necessary to develop new methodology that would satisfy these criteria and be sufficiently simple to be of value in large-scale plasma level studies.

The development of a new, specific assay method for hydralazine plasma levels and the results obtained in healthy human subjects by its use comprise the subject of this report.

## METHODS

**Protocol**—Healthy adult male volunteers participated in the studies after a complete medical history had been recorded for each individual. No medication was permitted for at least 3 days prior to the study. Control blood and urine specimens were obtained after an overnight fast and, subsequently, hydralazine was administered.

A pool of six volunteers participated in the studies involving nonradioactive hydralazine. A 100-mg oral dose, consisting of four 25-mg commercial tablets, was given to three subjects. A 75-mg oral dose (three 25-mg commercial tablets) was administered to three subjects on a different occasion. In addition, three individuals were given a 20-mg intramuscular dose of the commercial parenteral preparation. Certain individuals participated in more than one study; at least 1 week elapsed between successive experiments. The subjects were permitted to have a fat-free breakfast without coffee at 2 hr postadministration and resumed their normal eating habits at 5 hr.

In the study involving hydralazine-$^{14}$C, a 100-mg oral dose of the appropriately diluted radioactive material in capsules was administered to a different group of four subjects. These subjects had a simple fat-free meal without coffee at 4 hr postadministration and resumed their normal eating habits at 8 hr.

**Determination of Hydralazine Concentrations in Plasma by the Spectrophotometric ("Cold") Method**—Blood specimens (15 ml) were withdrawn with heparinized syringes prior to and at specified times after administration of hydralazine. They were transferred immediately to refrigerated, heparinized centrifuge tubes. The specimens were centrifuged in a refrigerated centrifuge, and the plasma was removed and mixed with 0.2 ml of 6 *N* HCl. This part of the procedure had to be completed within 40 min. The acidified specimens were frozen and stored in a freezer until they were analyzed. Under these conditions, hydralazine levels remained unchanged for 4 days.

Aliquots (6 ml) of plasma were transferred to a glass-stoppered centrifuge tube and diluted with 6 ml of 0.4 *N* HCl. To each sample, 0.2 ml of a 10% solution of ascorbic acid in water and 0.5 ml of a 1% solution of *p*-methoxybenzaldehyde in ethanol were added. The mixture was incubated for 30 min at 70°. The tubes were cooled to room temperature, and the pH was adjusted to 9.0 with 5 *N* NaOH. The resulting hydrazone derivative was extracted by shaking each tube on a mechanical shaker for 20 min with 30 ml of benzene–methanol (93:7). The organic phase was separated by centrifugation, and a 25-ml aliquot was transferred to another centrifuge tube. The solvent was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 3 ml of heptane and mixed with 1.5 ml of 0.2 *N* HCl. The hydrazone derivative was reextracted into the aqueous phase by shaking for 20 min. The mixture was centrifuged, the organic phase was removed by aspiration, and the aqueous phase was transferred with a Pasteur pipet into a microcell. The absorbance was read in a spectrophotometer[2] at 355 nm against 0.2 *N* HCl.

The value thus obtained was converted into concentration using a standard curve of solutions of authentic hydrazone derivative in 0.2 *N* HCl. To generate the standard curve, the concentrations in these solutions had to be determined without delay because breakdown of the hydrazone occurred after 20 min in 0.2 *N* HCl. However, in the presence of plasma extracts and excess reagents, the compound was stable for at least 60 min.

Plasma samples containing known amounts of hydralazine also were carried through the entire procedure along with the samples obtained in the study.

In the experiment using $^{14}$C-labeled hydralazine, 50-ml blood specimens were withdrawn. Plasma was obtained as described previously, and 6-ml aliquots were analyzed for hydralazine. The remaining amounts of plasma were used for measurement of radioactivity and for inverse isotope dilution analysis.

**Determination of Hydralazine Concentrations in Urine**—The spectrophotometric method described in detail for plasma was used with two modifications.

1. To 2 ml of urine were added 1 ml of 0.4 *N* HCl, 0.2 ml of a 10% aqueous solution of ascorbic acid, and 0.5 ml of a 1% ethanolic solution of *p*-methoxybenzaldehyde. Incubation at 70° and subsequent extraction at alkaline pH were carried out without modification.
2. Following the evaporation of the 25-ml aliquot of benzene–methanol extract to dryness, the residue was dissolved in 6 ml of heptane and reextracted with 3 ml of 0.2 *N* HCl. Subsequent steps were identical in the plasma and urine methods.

**Preparation of *p*-Methoxybenzaldehyde Hydrazone of Hydralazine**—To a solution of hydralazine (720 mg) in 7.5 ml of ethanol, 5 ml of water was added. The mixture was heated to 50°, and *p*-methoxybenzaldehyde (0.3 ml in 2.5 ml of ethanol) was added slowly with constant stirring. The resulting thick, yellow mass was stirred with an additional 5 ml of water. A few minutes later, 8 ml of 5% sodium bicarbonate was added and the mixture was cooled to room temperature. The precipitate was filtered, washed with ice-cold water and ethanol, and dried under reduced pressure. The crude product was recrystallized from 400 volumes of 60% aqueous methanol, giving the analytical sample, mp 130–132°; UV (0.1 *N* HCl containing 1% methanol): 299 and 358 nm ($\epsilon$ 23,300 and 31,200, respectively); UV (chloroform): 295 and 373 nm ($\epsilon$ 22,450 and 25,350, respectively); mass spectroscopy (mol. wt. 278): *m/e* 278, 277, 171 (base peak), 145, and 134; NMR (dimethyl sulfoxide-$d_6$): 3.8 (3H, s, $OCH_3$), 7.0 (2H, d, $J$ = 9 Hz, aromatic protons *ortho* to $OCH_3$), 7.8 (2H, d, $J$ = 9 Hz, aromatic protons *meta* to $OCH_3$), 8.42 (1H, s, heterocyclic ring proton), 7.5–8.5 (5H, multiplet, four aromatic protons of the bicyclic ring system and CH of the side chain), and 11.8 (1H, NH) ppm.

*Anal.*—Calc. for $C_{16}H_{14}N_4O$: C, 69.0; H, 5.1; N, 20.2. Found: C, 68.63; H, 5.27; N, 20.24.

**Preparation of Capsules Containing Hydralazine-$^{14}$C**—Hydralazine-1-$^{14}$C (specific activity 12.73 $\mu$Ci/mg) was obtained commercially[3]. The purity of the material was tested by TLC and measurement of radioactivity and was found satisfactory.

A mixture of 656.1 mg of unlabeled hydralazine and 43.9 mg of hydralazine-$^{14}$C was dissolved in 35 ml of water. The solution was freeze dried in a lyophilizer (Virtis), and the resulting powder was stored under nitrogen. Exactly 100-mg aliquots corresponding to 80 $\mu$Ci of $^{14}$C were transferred into commercial gelatin capsules for use in the plasma level studies.

**Determination of Total Radioactivity**—The plasma or urine concentration of total $^{14}$C isotope, corresponding to the sum of unchanged hydralazine and its metabolites, was measured by transferring appropriate aliquots to scintillation vials, adding 20 ml of Bray's solution (6), and counting in a liquid scintillation spectrometer[4]. Results were corrected for quenching by the external standard technique.

Feces were homogenized with water. The mixture was freeze

[2] Zeiss.
[3] Mallinckrodt Nuclear, St. Louis, Mo.
[4] Packard Tri-Carb model 3380.

**Table I**—TLC Behavior of Hydralazine and Its *p*-Methoxybenzaldehyde Hydrazone Derivative

| Solvent System | $R_f$ Value | |
|---|---|---|
| | Hydralazine | Hydrazone |
| Cyclohexane–acetone (5:4) | | 0.83 |
| Benzene–ethanol–acetic acid (220:10:1) | | 0.76 |
| Toluene–ethyl acetate (10:9) | | 0.73 |
| 3 *N* HCl–methanol–ascorbic acid (44:6:1) | 0.53[a] | |

[a] Hydralazine was spotted on methanol-washed, silica gel-coated glass plates under a current of nitrogen. The plates were developed in hydrochloric acid–methanol–ascorbic acid flushed with nitrogen before use. Considerable decomposition of hydralazine occurred on the plates when other solvent systems were used.

dried in a lyophilizer (Virtis), and aliquots of the dry residue were combusted in a sample oxidizer[5]. The resulting $^{14}C$-labeled carbon dioxide was collected in vials containing 1.5% of 2,5-diphenyloxazole and 0.1% of *p*-bis(*o*-methylstyryl)benzene in toluene and measured in the liquid scintillation spectrometer.

Plasma samples obtained from subjects who had been given hydralazine-$^{14}C$ were analyzed for unchanged hydralazine by the spectrophotometric method described previously. At the completion of these analyses, each sample was also assayed for its radioactivity content; appropriate aliquots were withdrawn and counted in the usual manner using Bray's solution.

**Determination of Hydralazine Concentrations in Plasma by Inverse Isotope Dilution Analysis**—The derivatization and extraction steps essentially followed the procedure outlined earlier in the spectrophotometric method for the assay of hydralazine. The few modifications were found advantageous because of the larger volume of plasma used.

Unlabeled hydralazine carrier (220 μg) was added to measured aliquots (13–14 ml) of plasma containing hydralazine-$^{14}C$ and its metabolites. Ascorbic acid (50 mg), 3 ml of 1 *N* HCl, 0.02 ml of a 10% ethanolic *p*-methoxybenzaldehyde solution, and finally 5 ml of ethanol were added to each plasma sample. The hydrazone derivative was formed by incubation of the mixture for 15 min at 65°. The tubes were cooled to room temperature, and the resulting hydrazone was extracted at pH 9 into benzene–methanol (93:7). The extract was concentrated to a small volume under a stream of nitrogen. The residue was applied in streaks to methanol-washed silica gel thin-layer plates and chromatographed.

The solvent systems suitable for the chromatography of hydralazine[6] and its *p*-methoxybenzaldehyde hydrazone, as well as the corresponding $R_f$ values, are summarized in Table I. The zone corresponding to the $R_f$ of the hydrazone was scraped off and eluted with ethyl acetate. The amount of the material present in the eluate was determined by measurement of its absorbance at 355 nm. The specific activity of the eluate was then calculated from measurement of its radioactivity content. The chromatography, elution, and measurements were repeated until a steady-state specific activity was obtained. Usually three or four cycles were carried out. Finally, the plasma concentration of hydralazine was estimated from the steady-state specific activity values using Eq. 1:

$$C_p\ (\mu g/ml) = \frac{C_c \times SA_{obs}}{SA_{adm}} \qquad \text{(Eq. 1)}$$

where $C_p$ is the plasma concentration of hydralazine before dilution with carrier, $C_c$ is the concentration of the carrier in the plasma to which it was added, $SA_{obs}$ is the observed steady-state specific activity of the purified sample, and $SA_{adm}$ is the specific activity of the administered drug.

## RESULTS

**Development of Improved Assay Method**—The condensation of the hydrazino side chain of hydralazine with several aromatic aldehydes (cinnamaldehyde, salicylaldehyde, 3,4,5-trimethoxybenzaldehyde, and 1-naphthaldehyde) was explored to find an improved assay method for the drug. Unfortunately, none of these derivatives had a markedly higher absorptivity in either acidic or alkaline solution than the *p*-hydroxybenzaldehyde hydrazone. The derivative that had relatively the highest absorptivity with the lowest plasma blank values was the reaction product with *p*-methoxybenzaldehyde. In addition, its extractability, compared to that of the corresponding *p*-hydroxy derivative, was advantageous for permitting further refinements in the methodology. Thus, the *p*-methoxybenzaldehyde hydrazone became the derivative of choice.

The specific improvements over Schulert's method (4) included evaporation of the solvent used for the initial extraction of the hydrazone derivative from plasma, dissolution of the residue in the nonpolar heptane, and reextraction of the hydrazone into aqueous hydrochloric acid solution. A considerable amount of interfering material was eliminated through these purification steps without causing an appreciable loss of the desired product. Changes in the pH of the reaction and extractions led to a greater stability of the resulting color and increased the overall reproducibility. Therefore, when this new procedure was used for analyses of plasma samples containing 0.1 μg/ml of hydralazine, absorbance values greater than two times plasma blank readings were obtained. The absorbance values were proportional to the concentration in the 0.1–5.0-μg/ml range.

Plasma containing known amounts of hydralazine gave the following absorbance values (average ± *SE*): 0.15 μg/ml, 0.055 ± 0.0003; 0.25 μg/ml, 0.089 ± 0.0012; 0.5 μg/ml, 0.177 ± 0.0026; and 1.0 μg/ml, 0.364 ± 0.0037. The values represent 38 determinations

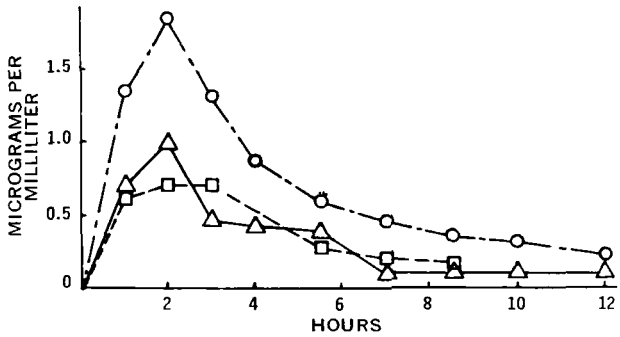


**Figure 1**—*Concentration of hydralazine-$^{14}C$ and its metabolites in the plasma of Subject 1 after an oral dose of 100 mg (80 μCi). Key: ○, total radioactivity; △, unchanged hydralazine by cold method; and □, unchanged hydralazine by inverse isotope dilution analysis.*

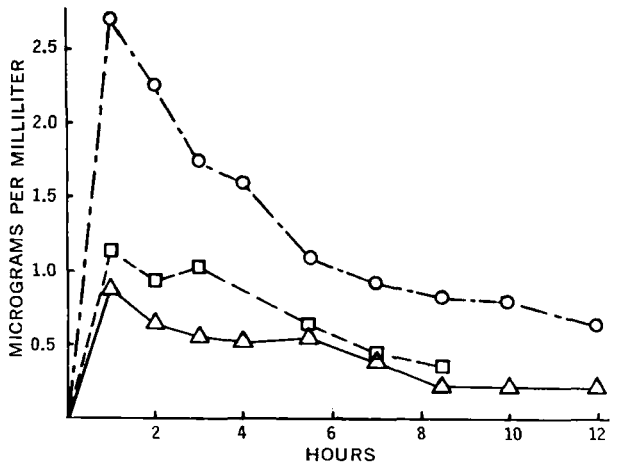


**Figure 2**—*Concentration of hydralazine-$^{14}C$ and its metabolites in the plasma of Subject 2 after an oral dose of 100 mg (80 μCi). Key: ○, total radioactivity; △, unchanged hydralazine by cold method; and □, unchanged hydralazine by inverse isotope dilution analysis.*

[5] Packard Tri-Carb.

[6] J. E. Francis, Ciba-Geigy Corp., Ardsley, N.Y., personal communication.

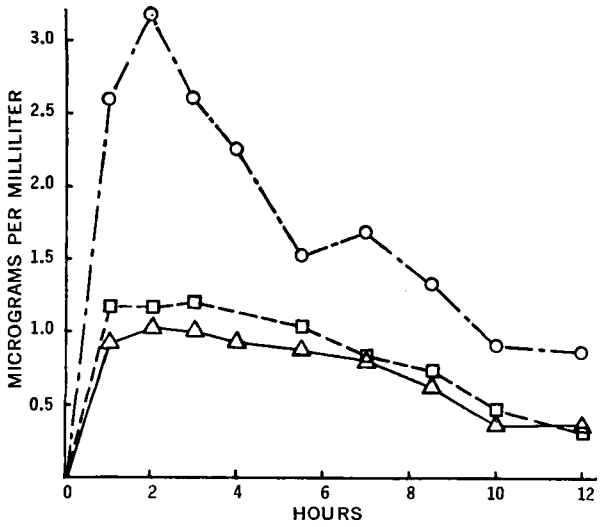


**Figure 3**—*Concentration of hydralazine-$^{14}C$ and its metabolites in the plasma of Subject 3 after an oral dose of 100 mg (80 μCi). Key: ○, total radioactivity; △, unchanged hydralazine by cold method; and □, unchanged hydralazine by inverse isotope dilution analysis.*

at each concentration after subtraction of the individual blank readings. The plasma blanks had an average absorbance value of 0.038 ± 0.0025. Recoveries, calculated from the 38 measurements, were (average ± *SE*): 96.0 ± 1.6%, 0.15 μg/ml; and 98.0 ± 1.0%, 1.0 μg/ml. The individual recovery values closely approximated a normal distribution at both concentrations (expected value of Pearson's coefficient of skewness: 0.0; found, 0.32 and 0.45, respectively; expected value of the moment coefficient of kurtosis: 3.0; found, 2.56 and 2.94, respectively). These data demonstrate the good reproducibility of the assay method.

Schulert recommended that blood specimens containing hydralazine be processed at a weakly acidic pH (6.5) in view of the instability of the free base form of the drug. In the present experiments, some degree of hemolysis could not be prevented under these conditions and the resulting pink color of the plasma interfered with the analysis, particularly at low drug concentrations. Therefore, it was necessary to study the stability of hydralazine in whole blood. At room temperature, hydralazine (0.25 μg/ml) was stable in plasma at pH 7 for 20 min but appreciable losses were encountered after storage for 30 min. However, in refrigerated, heparinized human whole blood the drug was stable for at least 40 min. Based on this finding, it was concluded that it was not necessary to acidify the blood before separation of the plasma provided that the specimens were cooled to 0° immediately after withdrawal and centrifuged in a refrigerated centrifuge and the plasma samples were acidified within 40 min from withdrawal.

In acidified human urine, hydralazine was stable for 60 min at room temperature and for at least 2 weeks at −20°.

**Plasma Levels of Hydralazine**—In preliminary studies, hydralazine was administered to groups of healthy individuals. Plasma levels of hydralazine were measured after oral administration of 100 mg to three subjects. Absorption of the drug was

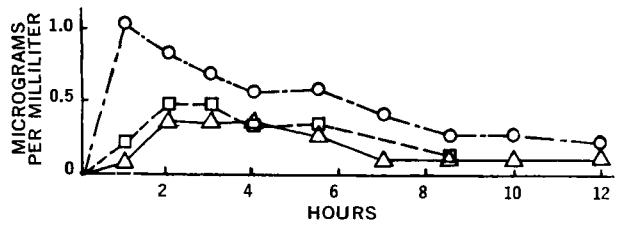


**Figure 4**—*Concentration of hydralazine-$^{14}C$ and its metabolites in the plasma of Subject 4 after an oral dose of 100 mg (80 μCi). Key: ○, total radioactivity; △, unchanged hydralazine by cold method; and □, unchanged hydralazine by inverse isotope dilution analysis.*

**Table II**—Excretion of Hydralazine-$^{14}C$ and Metabolites after a 100-mg Oral Dose

| Time Interval, hr | Cumulative Excretion of Radioactivity, % of Dose | | | |
|---|---|---|---|---|
| | Subject 1 | Subject 2 | Subject 3 | Subject 4 |
| Urine: | | | | |
| 0–4 | 49.1 | 40.4 | 33.1 | 5.5 |
| 0–8 | 50.8 | 60.5 | 54.6 | 30.8 |
| 0–12 | 54.7 | 68.0 | 62.6 | 43.4 |
| 0–24 | NA[a] | 76.5 | 73.9 | 49.6 |
| 0–48 | 55.8[a] | 78.5 | 79.7 | 50.9 |
| 0–72 | 56.0[a] | 80.9 | 80.9 | 51.5 |
| Feces: | | | | |
| 0–48 | NA | NA | 2.9 | 11.9 |

[a] NA = not available. The 12–24-hr urine fraction of Subject 1 was not available for analysis. Consequently, subsequent data on that individual underestimate the true magnitude of cumulative excretion.

rapid in all cases, maximum concentration being attained at 1 hr postadministration. Peak levels were 0.76, 0.95, and 1.03 μg/ml. Subsequently, a rapid decline occurred; at 24 hr, hydralazine concentrations were below the limit of detection in all subjects.

Similarly, maximum plasma concentrations of unchanged hydralazine after the administration of 75 mg were attained rapidly, ranging between 0.7 and 0.8 μg/ml. The limited amount of data of the experiments with the 100- and 75-mg doses represents a fairly good correlation between dose and the resulting peak levels. Individual half-lives could not be determined accurately in these experiments because of the fluctuating pattern of plasma levels. The appearance of secondary peaks may be related to biliary recirculation or changes in the volume of distribution of hydralazine. The available data are insufficient to permit a better understanding of these phenomena.

When hydralazine (20 mg) was administered intramuscularly to a group of three subjects, maximum concentrations of 0.16–0.61 μg/ml of the drug were attained within 1 hr. The fluctuating pattern of the plasma levels after the parenteral dose indicated that the intersubject differences and the occasional appearance of secondary peaks observed in the oral studies were probably not associated with a variability of GI absorption.

**Confirmation of Specificity of Assay Method in Human Studies**—An experiment was designed to assess the degree of

**Table III**—Urinary Excretion of Hydralazine

| Dose and Route | Subject | Time Interval, hr | Cumulative Excretion, % of Dose |
|---|---|---|---|
| 100 mg po | B.B. | 0–6 | 9.0 |
| | | 0–10 | 10.2 |
| | | 0–24 | 10.5 |
| | M.C. | 0–6 | 6.6 |
| | | 0–10 | 6.9 |
| | | 0–24 | 7.3 |
| | R.L. | 0–6 | 3.7 |
| | | 0–10 | 3.7 |
| | | 0–24 | 3.7 |
| 75 mg po | B.B. | 0–6 | 9.9 |
| | | 0–12 | 10.7 |
| | | 0–24 | 12.9 |
| | S.T. | 0–6 | 8.4 |
| | | 0–12 | 8.4 |
| | | 0–24 | 8.4 |
| | K.S. | 0–6 | 3.0 |
| | | 0–12 | 3.1 |
| | | 0–24 | 3.7 |
| 20 mg im | B.B. | 0–4 | 8.2 |
| | | 0–8 | 9.6 |
| | | 0–12 | 9.6 |
| | B.C. | 0–4 | 5.7 |
| | | 0–8 | 7.7 |
| | | 0–12 | 7.7 |
| | K.S. | 0–4 | 10.6 |
| | | 0–8 | 12.3 |
| | | 0–12 | 13.8 |

specificity of the new method (hereafter called the "cold" method). Four individuals were each given a 100-mg oral dose of hydralazine-$^{14}$C (80 μCi). Figures 1–4 show the time course of plasma levels of total radioactivity and unchanged hydralazine in these subjects. In all four cases, the plasma levels of total radioactivity, representing the sum of unchanged drug and its metabolites, reached maxima within 2 hr postadministration. These peak values ranged between 1.6 and 3.2 μg/ml, expressed as the amount of radioactivity that would correspond to unchanged hydralazine. The levels declined slowly and were still measurable at 12 hr postadministration. In each instance, higher levels were found for total radioactivity than for unchanged drug, indicating that hydralazine underwent extensive metabolism.

Some levels of unchanged hydralazine were measured by the cold method. Peak plasma levels were attained in all four individuals in 1–2 hr postadministration, indicating rapid GI absorption. In three of the four subjects, these peak levels ranged between 0.9 and 1.3 μg/ml. These values are comparable to the ones found after an oral dose of 100 mg of nonradioactive hydralazine. However, in the fourth subject, peak levels reached only 0.38 μg/ml. The levels declined rapidly, and in Subjects 1 and 4 the levels were not detectable after 5.5 hr postadministration. In Subjects 2 and 3, the decline was less rapid and a half-life of 6–8 hr could be estimated, although the measured values did not fall exactly on a monoexponential curve.

After each sample had been carried through the various purification steps of the cold method and their absorbance values had been determined, the radioactivity content of the solutions was also measured. The values obtained by the assay of radioactivity were essentially superimposable on those obtained by the cold method. The close agreement shows that the material carried through the cold method and assayed as apparent hydralazine did not contain appreciable amounts of nonradioactive endogenous compounds absorbing at the wavelength employed.

Analysis of each sample by the cold method and by determination of its radioactivity content could not exclude the possibility of interference by possible metabolites of hydralazine that might have retained the $^{14}$C-label and contributed to the UV readings. Therefore, as a definitive proof of the specificity of the cold method, the assay of most plasma samples was repeated by inverse isotope dilution analysis. The latter method is highly specific because it incorporates multiple TLC, thereby separating the hydrazone derivative of hydralazine from compounds that might affect the results obtained during the cold method. The results of these determinations are also shown in Figs. 1–4. The excellent agreement between the data obtained by the cold method and by inverse isotope dilution analysis demonstrates the specificity of the cold procedure.

**Excretion of Hydralazine and Its Metabolites**—Urinary excretion of radioactivity was measured in the four subjects administered 100 mg orally of hydralazine-$^{14}$C (Table II). In 72 hr, 51–81% of the dose was excreted, with most excretion taking place during the first 24 hr. Feces collected over 48 hr in two of the four subjects contained 2.9 and 11.9% of the dose, respectively. The findings of the urinary and fecal excretion pattern were confirmed recently[7].

At variance with the recent observations of Lesser *et al.* (7), unchanged hydralazine comprised only a small fraction of the urinary radioactivity, ranging from 3 to 14% of the dose. Similar results were obtained in the studies using nonradioactive hydralazine, the urinary excretion of unchanged drug ranging from 3.7 to 13.8% of the dose (Table III).

## DISCUSSION

The biological disposition of the antihypertensive drug hydralazine in man has not been the subject of detailed examination. Previous studies had been confined to hypertensive patients and mostly involved chronic administration of high doses. The primary objective of the present work was to obtain information on the biological disposition of hydralazine in healthy subjects after administration of a single therapeutic dose.

The analytical methodology developed in this laboratory has sufficient sensitivity for the determination of plasma levels of hydralazine after single oral therapeutic doses. Furthermore, it was demonstrated with the aid of inverse isotope dilution analysis that the method is specific for the assay of unchanged hydralazine in the presence of its metabolites.

The rate of decline of hydralazine plasma levels showed a marked intersubject variation. Fluctuating plasma levels and the occasional appearance of secondary peaks made the exact determination of the half-lives difficult. Nevertheless, in certain individuals the half-life was estimated to be in the 2–4-hr range whereas in others it approximated 6–8 hr. Pharmacogenetic factors may be partly responsible for the observed differences. Acetylation, a pathway subject to genetic control (8), has been known to constitute one *in vivo* metabolic transformation of hydralazine (1). Statistically significant differences in the average 2-hr plasma levels between genetically fast and slow acetylator individuals have been reported (5). Unfortunately, the latter study was limited to the 2-hr plasma levels, and the data do not lend themselves to conclusions regarding the time course of hydralazine in plasma. To the extent that acetylation represents a major metabolic pathway of hydralazine in man, genetic differences could conceivably affect the half-life of the drug. However, according to recent findings in these laboratories[8] and by Reidenberg *et al.* (9), the half-life of hydralazine does not necessarily correlate with the acetylator phenotype.

The data obtained in the present investigation describe the biological disposition of hydralazine in 10 healthy subjects. The results indicate rapid absorption and elimination; the latter process occurs primarily by metabolic transformation[8] of the drug and excretion of the metabolites mainly in the urine.

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