Colorimetric Determination of C-10 Hydroxylated Metabolites of Cyheptamide: Application to Absorption and Enzyme Induction Studies in the Rat

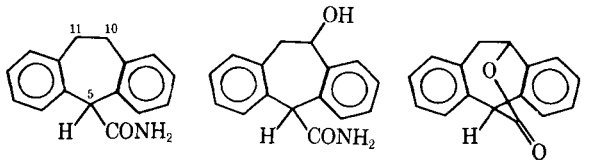
M. KRAML▲, D. DVORNIK, and L. COSYNS

Abstract □ A colorimetric procedure was developed for the quantitative estimation of some C-10 hydroxylated derivatives of cyheptamide and the lactones derived by acid-catalyzed rearrangement. The reproducibility, recovery, and specificity of the method were studied. In acid-hydrolyzed samples of human urine, "lactone" is regularly found with varying amounts of 5-hydroxy-lactone, and a suitable differential determination was also elaborated. By using this technique, it was demonstrated that in the rat, cyheptamide undergoes limited absorption which can be enhanced by micronization. Phenobarbital and primidone pretreatment enhances the C-10 hydroxylation of cyheptamide in the rat. Cyheptamide does not enhance its own catabolism.

Keyphrases □ Cyheptamide—absorption, catabolism, metabolites (colorimetric analysis), rats □ 10,11-Dihydro-10,5-(epoxymethano)-5*H*-dibenzo[*a*,*d*]cycloheptene-13-one—as cyheptamide metabolite, colorimetric analysis □ Absorption, cyheptamide—effect of micronization, rats □ Catabolism, cyheptamide—effect of drug-metabolizing enzyme, rats □ Colorimetry—analysis, cyheptamide metabolites

The anticonvulsant cyheptamide (1, 2) (AY-8682, 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5-carboxamide) (Scheme I) undergoes rapid and extensive metabolism in several species of laboratory animals (3). While the structures of all the metabolites remain to be elucidated, studies reported so far have established C-10 hydroxylation as an important metabolic transformation in rats, dogs, rabbits, and humans receiving cyheptamide. This conclusion was based on the isolation from acid-treated urine of 10,11-dihydro-10,5-(epoxymethano)-5*H*-dibenzo[*a*,*d*]cycloheptene-13-one (the "lactone"[1]). It was demonstrated (3) that the lactone is not a true metabolite of cyheptamide but is formed by acid hydrolysis of *syn*-10-hydroxycyheptamide or of some conjugate.

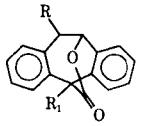
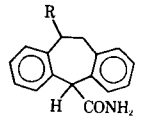
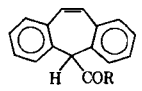
Projected pharmacokinetic studies, based on the analysis of cyheptamide itself, could not be done because the molecule does not contain any moiety through which a sensitive colorimetric or fluorometric assay could be developed. However, a colorimetric procedure, suitable for the determination of C-10 hydroxylated metabolites of cyheptamide, was developed. It depends on the ability of the lactone to form a stable chromogen in 30 *N* H_2SO_4. This method was applied to a study of the absorption of cyheptamide and also to a study of the effect of drug-metabolizing enzyme inducers on its catabolism in the rat.



Scheme I—Origin of lactone in acid-hydrolyzed urine of animals receiving cyheptamide

[1] The term "lactone" is used in this paper to refer exclusively to 10,11-dihydro-10,5-(epoxymethano)-5*H*-dibenzo[*a*,*d*]cycloheptene-13-one.

Table I—Specificity of Lactone Determination

Formula	Trivial Name	Color Yield[a] A	Color Yield[a] B
	Lactone, R = R_1 = H	100	100
	5-Hydroxy-lactone, R = H, R_1 = OH	145	145
	11-Hydroxy-lactone, R = OH (*syn*), R_1 = H	115	0
	11-Hydroxy-lactone, R = OH (*anti*), R_1 = H	115	0
	Cyheptamide, R = H	0	0
	10-Hydroxycyheptamide, R = OH (*syn*)	9	90
	10-Hydroxycyheptamide, R = OH (*anti*)	0	90
	AY-15,613, R = NH_2	0	0
	Triene acid, R = OH	98	4

[a] The color yield is determined at 528 nm. with lactone set arbitrarily at 100%. A = determined directly in 30 *N* H_2SO_4, and B = determined through the method.

MATERIALS AND METHODS

Reagents—The following reagents were used: (*a*) 5.0 *N* hydrochloric acid; (*b*) methylene chloride, reagent grade[2]; (*c*) activated charcoal[3]; (*d*) sodium sulfate anhydrous, analytical reagent; (*e*) silica gel[4], activated by heating overnight at 120° before use; (*f*) 30 *N* sulfuric acid; (*g*) lactone standard: a solution of authentic synthetic material (4) (300 mcg./ml.) in methylene chloride; (*h*) 5-hydroxy-lactone standard: a solution of authentic synthetic material (4) (200 mcg./ml.) in methylene chloride; and (*i*) other standard compounds: compounds structurally related to cyheptamide or lactone (3, 4) used to ascertain the specificity of the method (Table I).

Determination of Lactone—An aliquot of urine (2.0 ml.) is pipeted into a 40-ml. glass-stoppered centrifuge tube together with 1.0 ml. of 5 *N* HCl. The volume is completed to 5.0 ml. with water, and the tube is placed in a boiling water bath for 15 min. After cooling to room temperature, 10 ml. of methylene chloride is added and the tube is shaken vigorously for 15 min. The tube is centrifuged for 5 min. at 500 r.p.m. and the aqueous phase is siphoned off. A pinch (10–20 mg.) of activated charcoal and 0.5 g. of anhydrous sodium sulfate are added and the tube is shaken vigorously. An 8.0-ml. aliquot of the methylene chloride layer is passed through a short column[5] of silica gel. The column is rinsed with 5.0 ml. of methylene chloride, and the combined eluates are evaporated

[2] Fisher.
[3] Norit SG Extra, American Norit Co.
[4] Grace, Grade 923.
[5] A microcolumn, 50 mm. in length with an internal diameter of 5 mm., equipped with a reservoir of 15-ml. capacity, was employed.

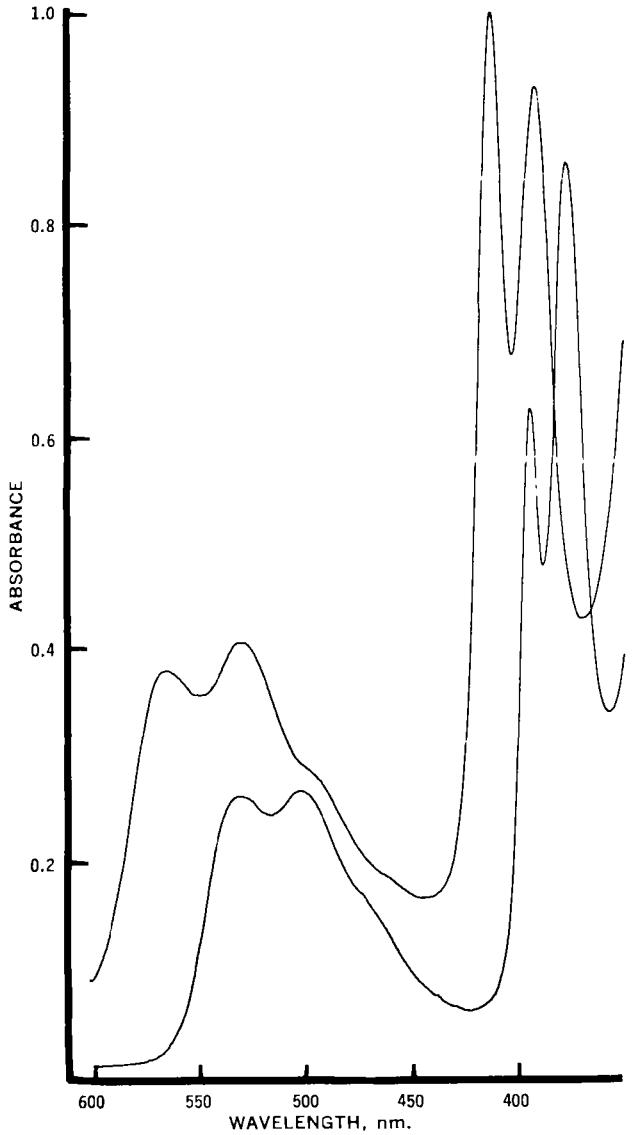


Figure 1- *Visible spectra of lactone (lower tracing) and 5-hydroxy-lactone (upper tracing) in 30* N H_2SO_4.

to dryness on a boiling water bath. Then 4.0 ml. of 30 *N* H_2SO_4 is added to the residue and the tube is mixed vigorously with a mixer (Vortex). Color is developed by heating for 15 min. in a boiling water bath, and it is read at 528 nm. in a spectrophotometer (Unicam). Urine blanks are carried through the same procedure. Standards (300 mcg.) of pure lactone are concurrently run in triplicate.

Simultaneous Determination of Lactone and Its 5-Hydroxy Derivative—The 5-hydroxy-lactone is eluted from the silica gel column together with the lactone. However, on reaction with 30 *N* H_2SO_4, the former gives a spectrum with a hyperchromic shift of 20 40 nm. Thus, by reading the optical density at two wavelengths--*viz.*, at 528 and 564 nm., a differential determination of the lactone and its 5-hydroxy derivative can be carried out.

Biological Methods—In these studies, two forms of cyheptamide were used: (*a*) the usual coarse material with particle size of approximately 500 μ, and (*b*) a fine-particle preparation[6] with 85% of the particles smaller than 10 μ, as measured in a counter (Coulter). In some experiments, $^{14}CONH_2$-cyheptamide (3) was also employed. Lactones were estimated as already described, and in experiments with ^{14}C-labeled cyheptamide, total urinary metabolites were estimated by counting 0.2-ml. aliquots of urine in dioxane fluor using a liquid scintillation system[7].

In all experiments, cyheptamide was administered by gavage as 1.0 ml. of a saline suspension for 100 g. body weight. The animals, supplied only with drinking water, were then placed in individual stainless steel metabolism cages; the 24-hr. urines were collected and, together with cage washings, were completed to 25 ml. Aliquots of 5.0 ml. were used in the colorimetric assays.

Absorption Studies—Both coarse cyheptamide and micronized cyheptamide were administered orally to male albino rats, eight per group, weighing about 150 g. each, and the relative degree of absorption was determined by measuring the lactone in the 24-hr. urine samples. Excretion of cyheptamide metabolites in the rat is completed in less than 24 hr. (3). Doses of 50, 100, and 500 mg./kg. were employed. To use the excretion of a metabolite as an index of drug absorption, it must be demonstrated that the metabolite, under standard conditions, accounts for the constant amount of the total urinary metabolites. To this end a group of 10 male albino rats were given, by gavage, 100 mg./kg. of $^{14}CONH_2$-cyheptamide (undetermined particle size), and both the lactone and the total urinary metabolites were estimated in the 24-hr. urine samples.

Drug-Metabolizing Enzyme Induction Studies—Induction of hepatic enzymes was assessed by measuring the ability of the various agents to affect the extent of benzylic (C-10) hydroxylation of cyheptamide. Increased urinary excretion of C-10 hydroxylated metabolites of cyheptamide by rats pretreated with anticonvulsants (above the levels of the saline controls) after a challenge dose of cyheptamide would be indicative of enzyme induction caused by these agents. Thus male albino rats, eight per group, weighing about 150 g. each, were given a daily oral dose of 25 mg./kg. of either phenobarbital, primidone, or cyheptamide. After 4 weeks, all animals, including the saline controls, were given an oral challenge dose of 100 mg./kg. of cyheptamide and the 24-hr. urines were collected. Urinary C-10 hydroxylated metabolites were determined as lactones (in this study, both lactone and 5-hydroxy-lactone were determined).

In experiments where the rats received a daily dose of cyheptamide, it was necessary to run an extra set of controls. On the last

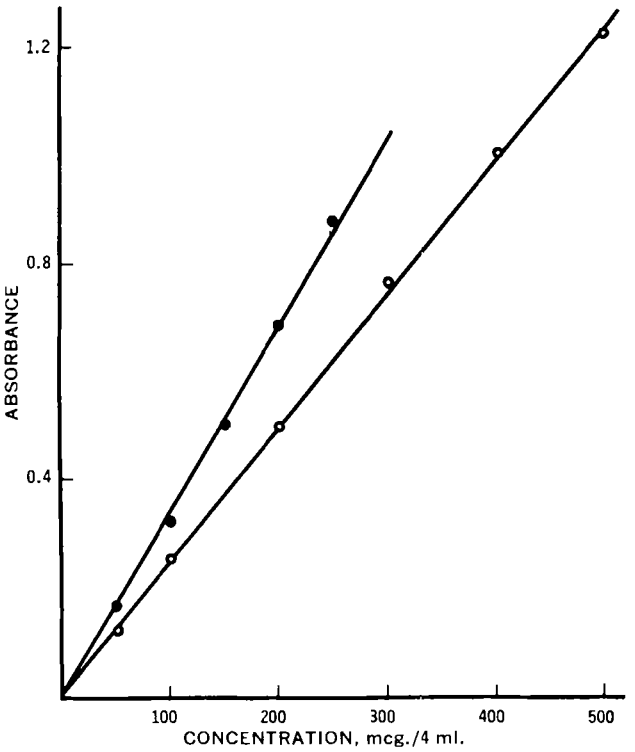


Figure 2—*Standard curves of lactone (528 nm.)* (○) *and 5-hydroxy-lactone (564 nm.)* (●) *in 30* N H_2SO_4.

[6] Courtesy of Dr. D. Beall and Dr. J. Devaney, Product Development Laboratories, Ayerst, Rouses Point, N.Y.

[7] Nuclear Chicago model 720.

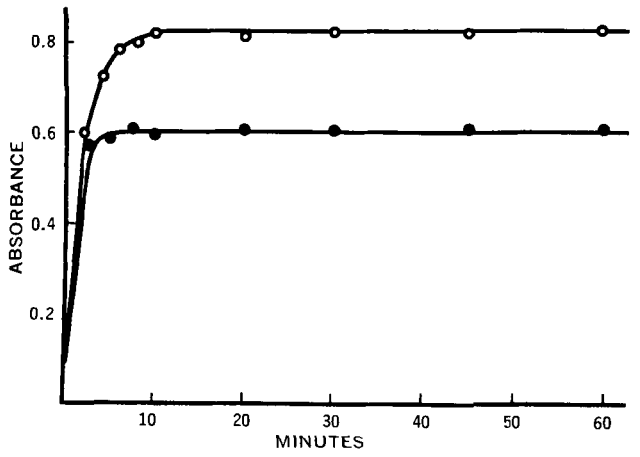


Figure 3—*Effect of time on rate of color development of lactone* (○) *(read at 528 nm.) and 5-hydroxy-lactone* (●) *(read at 564 nm.), on heating at 100° in 30* N H_2SO_4.

day, these animals did not receive the 100-mg./kg. challenge dose. The small amounts of C-10 hydroxylated metabolites contributed by the daily 25-mg./kg. dose of cyheptamide were estimated and subtracted from the values of the test group.

RESULTS

Spectral Data—The spectra obtained for the lactone and for its 5-hydroxy derivative in 30 *N* H_2SO_4 are presented in Fig. 1. With the 5-hydroxy-lactone, all peaks are shifted to higher wavelengths by approximately 40 nm.

The colored molecular species produced on treatment of the lactone with strong acid were identified as dibenzotropylium ions. Their structures and the mechanism of their generation are presented elsewhere (5).

Adherence to Beer's Law—Standard curves for the lactone and its 5-hydroxy derivative are presented in Fig. 2. Strict adherence to Beer's law was obtained with the lactone between 50 and 500 mcg. and with its 5-hydroxy derivative between 25 and 250 mcg./tube. For 100 mcg., lactone at 528 nm. gives an average absorbance of 0.247 ± 0.003, while its 5-hydroxy derivative, at 564 nm., gives an average absorbance of 0.327 ± 0.003. Qualitatively, when a urine sample contains a mixture of both lactones, the presence of the 5-hydroxy-lactone is indicated by the appearance of a cherry tint superimposed on the normal orange color produced by treatment with sulfuric acid.

Rate of Color Development in 30 *N* H_2SO_4—Lactone (300 mcg.) was dried in a series of tall test tubes; to each tube was added 4.0 ml. of 30 *N* H_2SO_4. The tubes were heated at 100° in a boiling water bath for varying times, from 2 to 60 min. The tubes were then plunged into ice water to stop color development and were read in a spectrophotometer (Unicam) at 528 nm. The same procedure was followed with the 5-hydroxy-lactone, but the color was read at 564 nm. The results are plotted in Fig. 3. At 10 min., color development is complete and a plateau is maintained up to 60 min. The same samples were read after standing at room temperature for 24 hr., and no change in absorbance was noted. Thus, in 30 *N* H_2SO_4 both lactones rapidly form stable chromogens.

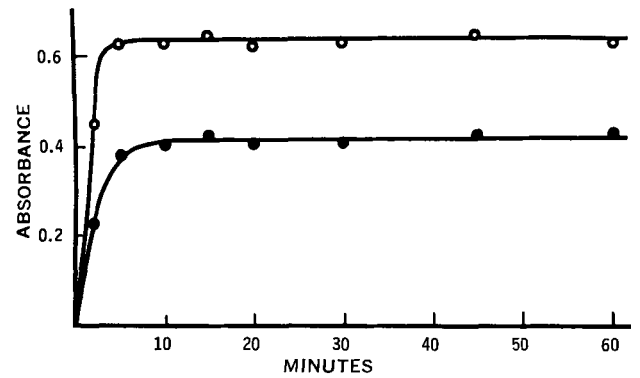


Figure 4—*Effect of time on the acid hydrolysis (in 1* N *HCl at 100°) of* anti*-10-hydroxycyheptamide* (○) *and cyheptamide metabolites in urine* (●) *to lactone.*

Rate of Lactone Formation by Hydrolysis—The *syn*-10-hydroxycyheptamide and/or its conjugate(s) produce a characteristic color with 30 *N* H_2SO_4 only after they are treated with acid to produce the lactone. This procedure is routinely carried out in 1 *N* HCl at 100° in a boiling water bath. The effect of duration of hydrolysis was investigated with an authentic sample of synthetic *anti*-10-hydroxycyheptamide and with a urine sample known to contain conjugated 10-hydroxylated metabolites of cyheptamide. Samples of 10-hydroxycyheptamide (300 mcg.) and of urine (2.0 ml.) were hydrolyzed in 1 *N* HCl at 100° for times varying from 2 to 60 min. and were analyzed as already described. Data presented in Fig. 4 show that hydrolysis is complete in 10 min. and remains unchanged during periods up to 60 min. The yield of conversion of 10-hydroxycyheptamide[8] to the lactone in three separate experiments was 89.8 ± 1.1%. Since authentic 5,10-dihydroxycyheptamide was not available, its conversion to the corresponding lactone was not studied. It is assumed that the conditions verified with 10-hydroxycyheptamide are also suitable for its 5,10-dihydroxy analog.

Recoveries of Lactone—Varying amounts of lactone (100–500 mcg.) or its 5-hydroxy derivative (50–250 mcg.) were added to water and urine and carried through the entire procedure as previously described. Recoveries were calculated based on test tube standards run simultaneously. Recoveries of lactone were 90.0–97.4% (93.6 ± 0.2%) from water and 83.4–94.5% (89.6 ± 0.4%) from urine. With the 5-hydroxy-lactone, the recoveries were 90.5–98.5% (94.8 ± 2.1%) from urine. Seven separate experiments were performed. Losses due to partition between methylene chloride and water, absorption on charcoal, or retention on the silica gel are minimal. Recoveries were not concentration dependent, being equal throughout the range studied.

Urine Blanks—When attempts were made to determine lactone levels in urine samples without charcoal treatment or silica gel chromatography, blanks with absorbances of 0.500 and higher were obtained. Usually, the chromatography on silica gel eliminated most of these impurities; however, some urine samples, after hydrolysis, contained great amounts of pigments, some of which passed through the column. A pinch of activated charcoal removed most of these impurities. Activated charcoal alone, while decreasing blank values, did not lower them to acceptable levels and the combination with silica gel chromatography was necessary. With the method as described here, the average absorbance of blanks obtained with human or rat urine was 0.030 ± 0.002 (15 samples) at 528 nm. and 0.020 ± 0.003 at 564 nm. Calculated as lactone(s), this corresponds to a level of approximately 0.5 mg./100 ml.

Specificity of Method—Compounds structurally related to cyheptamide and to the lactone were tested for their possible interference with the colorimetric determination of the lactone. Two types of experiments were performed: "direct" interference was measured by estimation of the color yield obtained with the compounds in 30 *N* H_2SO_4, and "true" interference was measured by estimation of the color yield obtained with the compounds after treatment with acid and processing through the entire method. The results are presented in Table I.

With 30 *N* H_2SO_4, only 5*H*-dibenzo[*a*,*d*]cycloheptene-5-carboxylic acid (triene acid) gave the same chromogen as the lactone. On a molar basis, the color yield is virtually the same. However, carried through the method, the triene acid does not interfere since all but approximately 4% is retained by silica gel.

Both the *syn*- and *anti*-11-hydroxy-lactones gave stable green chromogens which interfere with the lactone determination at 528 nm. to the extent of 115%. However, when carried through the method, the 11-hydroxy-lactones are quantitatively retained by silica gel, thus eliminating their interference.

5-Hydroxy-lactone gives an intensely colored chromogen with 30 *N* H_2SO_4; inasmuch as it is eluted from silica gel in the same frac-

[8] The *syn*-10-hydroxycyheptamide was too unstable to use as a colorimetric standard. Its acid-catalyzed conversion to the lactone is even more rapid than that of the *anti*-isomer.

Table II—Differential[a] Determination of Lactone and 5-Hydroxy-Lactone in Mixtures

Sample Number	Lactone, mcg./ml. Calc.	Lactone, mcg./ml. Found	Lactone, Percent Recovered	5-Hydroxy-Lactone, mcg./ml. Calc.	5-Hydroxy-Lactone, mcg./ml. Found	5-Hydroxy-Lactone, Percent Recovered
1	200	200.0	100.0	0	0	—
2	175	171.6	98.0	25	22.7	90.8
3	150	150.9	100.6	50	45.3	90.6
4	125	124.9	99.9	75	70.4	93.8
5	100	102.2	102.2	100	94.9	94.9
6	75	76.7	102.2	125	119.6	95.6
7	50	48.6	97.2	150	147.6	98.4
8	25	22.6	90.4	175	173.0	98.8
9	0	0		200	200.0	100.0
Average		98.6 ± 1.6%			94.7 ± 1.4%	

[a] Measured at 528 and 564 nm. and calculated from the equations:

$$\text{lactone (mcg.)} = \frac{(B')(b)(X) - (B)(b)(X')}{(A)(B') - (A')(B)}$$

$$\text{5-hydroxy-lactone (mcg.)} = \frac{(A)(a)(X') - (A')(a)(X)}{(A)(B') - (A')(B)}$$

where A, B, and X and A', B', and X' are the absorbances of lactone, 5-hydroxy-lactone, and unknown at 528 and 564 nm., respectively; and a and b are the concentrations (micrograms per milliliter) of the lactone plus 5-hydroxy-lactone standards.

tion as the lactone, it causes an interference of 145%. Unlike the lactone, the 5-hydroxy-lactone has an absorption maximum at 564 nm. Using this, the contribution to lactone absorption at 528 nm. can be calculated.

anti-10-Hydroxycyheptamide gave no color on treatment with 30 N H_2SO_4. The value of 9% observed for the *syn*-isomer most likely represents lactone in the stock solution arising from the spontaneous lactonization of the extremely labile *syn*-isomer prior to addition of 30 N H_2SO_4. After acid hydrolysis and processing through the entire method, both the 10-hydroxycyheptamides gave a color yield of approximately 90%. Obviously, this represents the yield of conversion, upon acid hydrolysis, of the 10-hydroxy-cyheptamides to the lactone.

No other compound tested gave a color reaction when treated with 30 N H_2SO_4, either directly or after being carried through the method.

Differential Spectrophotometry—Mixtures of lactone and its 5-hydroxy derivative were prepared and analyzed by differential colorimetry at 528 and 564 nm. (*cf.*, Fig. 2). Solution of the simultaneous equations indicated (Table II) that in mixtures varying from 200 to 0 mcg. of lactone and from 0 to 200 mcg. of its 5-hydroxy derivative, the recoveries of lactone and 5-hydroxy-lactone were 98.6 ± 1.6 and 94.7 ± 1.4%, respectively.

Effect of Particle Size on Cyheptamide Absorption—When $^{14}CONH_2$-cyheptamide (particle size undefined) was administered orally to rats, an average of 28.7 ± 2.4% of the dose was excreted in the 24-hr. urine samples. Of this amount, 16.9 ± 1.5% was accounted for by C-10 hydroxylated metabolites. Thus, under a given set of conditions, the lactones do account for a consistent fraction of the urinary metabolites (and dose) of the orally administered cyheptamide and, in accordance with the suggestion of Smith *et al.* (6), the urinary C-10 hydroxylated metabolites can serve as a useful index of cyheptamide absorption.

This view is further strengthened by the data obtained on the urinary excretion of C-10 hydroxylated metabolites after the oral administration of graded doses of both coarse- and fine-particle cyheptamide to rats (Table III). With both preparations, within narrow limits, the 24-hr. lactone values were proportional to the dose.

Since cyheptamide is a neutral substance practically insoluble in water, it was expected that its absorption from the GI tract might be limited and would be enhanced by using micronized material as was, for example, demonstrated for griseofulvin (7, 8). This was indeed the case; at all dose levels studied, an approximately twofold enhancement (average value 2.2-fold, Table III) in urinary excretion of C-10 hydroxylated metabolites of cyheptamide was seen with the fine-particle material.

Table III—Effect of Dose and Particle Size[a] of Cyheptamide on the Urinary Levels of Its C-10 Hydroxylated Metabolites in the Rat[b]

Dose, mg./kg.	C-10 Hydroxylated Metabolites[c], mg./24-hr. Urine: Common Material	C-10 Hydroxylated Metabolites[c], mg./24-hr. Urine: Fine-Particle Preparation	Ratio, Fine/Common
50	0.18 ± 0.02 (1.0)[d]	0.35 ± 0.02 (1.0)	1.9
100	0.32 ± 0.02 (1.8)	0.94 ± 0.08 (2.7)	2.9
500	2.09 ± 0.24 (11.6)	3.60 ± 0.20 (10.2)	1.7

[a] Particle size of common material was approximately 500 μ; the fine preparation had 85% of particles smaller than 10 μ. [b] Male albino rats, weighing approximately 150 g., were used in groups of eight animals per dose. [c] Determined as lactone following acid hydrolysis. [d] Values *in parentheses* are ratios compared to the respective 50-mg./kg. doses.

Table IV—Effect of Chronic[a] Oral Administration of 25 mg./kg./day of Primidone or Phenobarbital on the Urinary Levels of C-10 Hydroxylated Metabolites of Cyheptamide[b]

Group	C-10 Hydroxylated Metabolites[c], mg./24-hr. Urine: Lactone	C-10 Hydroxylated Metabolites[c], mg./24-hr. Urine: 5-Hydroxy-Lactone
Control	1.13 ± 0.06	0.26 ± 0.02
Primidone	2.12 ± 0.20[d]	0.16 ± 0.03
Phenobarbital	2.78 ± 0.30[d]	0.15 ± 0.02

[a] Groups of eight male albino rats, weighing approximately 150 g. each, were treated for 4 weeks. [b] Each rat was given a single oral dose of 100 mg./kg. of cyheptamide, and urine was collected for 24 hr. [c] Lactones determined following acid hydrolysis. [d] $p < 0.01$.

This is indeed the case; at all dose levels studied, an approximately twofold enhancement (average value 2.2-fold, Table III) in urinary excretion of C-10 hydroxylated metabolites of cyheptamide was seen with the fine-particle material.

Enzyme Induction and C-10 Hydroxylation of Cyheptamide—The data on the excretion of urinary C-10 hydroxylated metabolites of cyheptamide by rats receiving a chronic administration of phenobarbital or primidone (25 mg./kg./day p.o. for 4 weeks), followed by a challenge dose of cyheptamide (100 mg./kg., by gavage), are presented in Table IV.

Chronic administration of either of these drugs provoked a more than 100% increase in the urinary excretion of C-10 hydroxylated metabolites of cyheptamide above the values for the saline-treated controls. 5,10-Hydroxylated metabolite levels remained unchanged.

The observed elevations are ascribed to increased enzymatic C-10 hydroxylation of cyheptamide induced by pretreatment with primidone and phenobarbital agents known to induce elevations in a number of microsomal, drug-metabolizing enzymes (9).

Chronic treatment of rats with cyheptamide did not affect the urinary levels of C-10 nor 5,10-hydroxylated metabolites of cyheptamide (Table V). Unlike primidone or phenobarbital, cyheptamide does not seem to induce the enzyme(s) involved in its C-10 hydroxylation in the rat.

DISCUSSION

A sensitive analytical method (other than an isotope method which would find only limited application in clinical studies) for cyheptamide is as yet not available. However, taking advantage of

Table V—Effect of Chronic[a] Oral Administration of 25 mg./kg./day of Cyheptamide on the Urinary Levels of Its C-10 Hydroxylated Metabolites

Group	10-Hydroxylated Metabolites[b], mg./24-hr. Urine: Lactone	10-Hydroxylated Metabolites[b], mg./24-hr. Urine: 5-Hydroxy-Lactone
Control	1.62 ± 0.17	0.47 ± 0.04
Cyheptamide[c]	1.71 ± 0.18	0.32 ± 0.05

[a] Groups of eight male albino rats, weighing approximately 150 g. each, received cyheptamide over 4 weeks. Subsequently, each control and treated rat was given a single oral dose of 100 mg./kg. of cyheptamide, and urine was collected for 24 hr. [b] Lactones determined following acid hydrolysis. [c] Values corrected for the contribution of the 25-mg./kg. dose of cyheptamide used in the chronic treatment period.

the metabolic conversion of cyheptamide to C-10 hydroxylated metabolites, a colorimetric method for these metabolites, based on the formation of a stable chromogen in 30 N H_2SO_4, was developed and applied to the analysis of urine samples of animals or humans. The method is specific and can detect as little as 50 mcg. of lactone (25 mcg. of 5-hydroxy-lactone). Attempts to estimate the levels of 10-hydroxylated metabolites of cyheptamide in the serum of laboratory animals (even at higher doses of cyheptamide) were not successful. This is not surprising in view of the low (1–15 mcg./ml.) serum levels of total cyheptamide metabolites found after administration of labeled material (3).

In the rat, the excretions of C-10 hydroxylated metabolites of cyheptamide were dose dependent, indicating their usefulness as an index of absorption. Fine-particle (micronized) cyheptamide in the same dose range gave a twofold increase in urinary C-10 hydroxylated metabolites, underlining the limited absorption of the coarse material. While the particle size of cyheptamide appears to play an important role in its absorption in the rat, it does not appear to be a critical factor with humans; after oral administration of $^{14}CONH_2$-cyheptamide (particle size not defined but most likely fairly coarse material), as much as 75% of the dose could be accounted for in the urine (3).

In rats pretreated with phenobarbital or primidone, enhanced catabolism (increased urinary C-10 hydroxylated metabolites) of cyheptamide is readily demonstrated. Cyheptamide itself does not possess this property to any significant degree.

Enhancement of drug-metabolizing enzymes by phenobarbital, *etc.*, is known to have profound effects on the circulating levels and clinical effectiveness of many other drugs (9). This fact may be of considerable importance in the clinical evaluation of cyheptamide where it is often administered to patients already receiving anticonvulsant (phenobarbital) therapy.

While the colorimetric determination of urinary C-10 hydroxylated metabolites cannot be used as an absolute measure of cyheptamide absorption, it should be of help in clinical trials to answer several questions. Are patients regularly taking the prescribed medication? Does the bioavailability of cyheptamide vary with different formulations? Is cyheptamide absorption irregular, and can patients be found who require more than the normal therapeutic dose? To answer these questions, it would obviously be preferable to determine cyheptamide (or metabolite) blood levels. At present this is not possible, although preliminary experiments indicated that the lactones do possess some fluorescence in 30 N H_2SO_4 and that this property could be the basis of a blood level method for C-10 hydroxylated metabolites of cyheptamide.

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▲ To whom inquiries should be directed.

A Method for Study of Timed-Release Films

NUTAN B. SHAH and BHOGI B. SHETH▲

Abstract □ A procedure for the *in vitro* evaluation of timed-release film compositions was investigated. The procedure consisted of measuring release rates of films cast from solutions of selected polymer compositions and physically examining the films. Films with varying proportions of ethylcellulose and hydroxypropyl methylcellulose were used as the model system. The effect of film thickness, film composition, and drying process was investigated. A special film holder was used for release rate studies. A high concentration solution of FD&C Red No. 2 was used as the model solute. Release rate studies were done in a rotating-bottle apparatus. Films were characterized for their release properties by measuring the rate of transport of the dye as the release rate constant, K. Quantitative data on variation in release characteristics as a function of the experimental parameters are presented. Microscopic examination showed maldistribution of hydrophilic polymer under certain drying conditions. However, release rates did not change appreciably with variation in the drying process under the conditions of this study.

Keyphrases □ Timed-release films—*in vitro* release rates, effect of thickness, composition, drying process □ Ethylcellulose films—*in vitro* release rates, effect of thickness, composition, drying process □ Hydroxypropyl methylcellulose films—*in vitro* release rates, effect of thickness, composition, drying process □ Release rates, ethylcellulose and hydroxypropyl methylcellulose films—effect of thickness, composition, drying process □ Film compositions, timed release—effect of thickness, composition, drying process on release rates

The use of barrier films, applied by film coating, is one of the more common methods used to formulate timed-release solid dosage forms. The barrier properties of film coatings are affected by formulation and processing parameters such as film composition, thickness, and drying conditions.

The normal course of development of such products requires several experimental products to be prepared