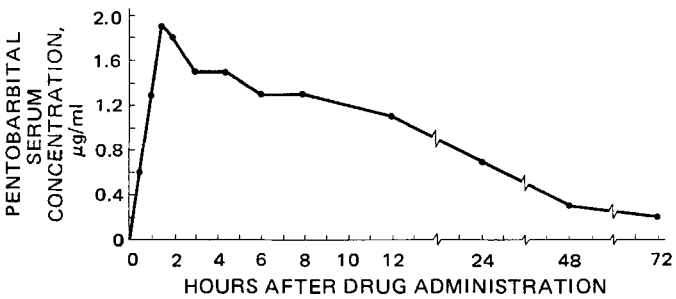

Figure 5—*Serum concentration of pentobarbital* versus *time in one human subject after single-dose oral administration of 100 mg of drug/capsule.*

injection (Fig. 1A). However, under the assay conditions, the lower limit of quantitation for I in extracts of human serum was 0.1 μg/ml, using 1 ml of serum sample (Table I). A linear relationship between peak area ratios and concentrations was obtained for I up to 1.0 μg/ml. Analyses of triplicate serum samples at 0.1–1.0 μg/ml gave a relative standard deviation of 2.6–16.5% with 1 μg of internal standard (Table I).

The method is specific for pentobarbital. Except for thiopental, it was resolved from 13 other barbiturates. The retention times for the pentafluorobenzyl derivatives of 15 barbiturates synthesized individually under similar conditions and detected by the electron-capture detector are shown in Table II. However, the derivatives did not resolve well when all 15 barbiturates were reacted simultaneously with pentafluorobenzyl bromide.

Serum Pentobarbital Levels in Humans—This analytical methodology was used for the measurement of serum pentobarbital (I) concentrations in normal human subjects after a single oral dose of 100 mg of pentobarbital sodium (capsule form). The subjects were fasted for a minimum of 12 hr prior to dosing and for 2 hr after dosing. The drug was administered with 120 ml (4 oz) of water.

Peak levels of pentobarbital ranged from 1.2 and 3.1 μg/ml and were observed at 0.5–2.0 hr after drug administration. Substantial amounts of drug (0.30 ± 0.08 μg/ml) were found in the 48-hr serum samples. A typical serum concentration *versus* time curve for one subject is shown in Fig. 5.

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[x] To whom inquiries should be directed.

In Vitro Adsorption of Acetaminophen onto Activated Charcoal

CAROL A. BAINBRIDGE [*], ERNEST L. KELLY [‡], and W. DOUGLAS WALKLING [x]

Abstract □ *In vitro* experiments supported *in vivo* evidence that activated charcoal is effective in adsorbing acetaminophen. In the physiologic pH range, adsorption was rapid and pH independent. Adsorption, however, was dependent upon the quantity of activated charcoal employed, becoming more complete as the quantity of activated charcoal was increased.

Keyphrases □ Acetaminophen—*in vitro* adsorption onto activated charcoal, effect of pH □ Adsorption, *in vitro*—acetaminophen onto activated charcoal, effect of pH □ Charcoal, activated—*in vitro* adsorption of acetaminophen, effect of pH □ Analgesics—acetaminophen, *in vitro* adsorption onto activated charcoal, effect of pH

The use of activated charcoal has been overlooked as an emergency procedure in acute acetaminophen overdose. Activated charcoal, given in powdered form, effectively decreased absorption of acetaminophen *in vivo* (1–3). When 2 g of acetaminophen was administered simultaneously with 10 g of activated charcoal suspended in a

Table I—Percent Acetaminophen Adsorbed onto Activated Charcoal from Two Sources[a] at pH 6.0 and 37.5°

Activated Charcoal, g[b]	Percent Adsorbed	
	Powder 1	Powder 2
0.3	53.7	65.2
0.4	68.0	86.1
0.5	81.2	93.8
0.6	90.0	97.1
0.7	95.9	98.9
1.0	99.0	99.8

[a] Powder 1 was Norit A, Matheson, Coleman and Bell, Norwood, Ohio. Powder 2 was from Amend Drug and Chemical Co., New York, N.Y. [b] Quantities per 50 ml of acetaminophen solution, 2.5 mg/ml.

methylcellulose solution, absorption in humans was reduced by 63% (1). When activated charcoal was given 60 min after acetaminophen ingestion, a 23% reduction in absorption was noted. When 10 g of activated charcoal was administered immediately after a 1-g dose of acetaminophen, a reduction of 69–77% was observed (2). Activated charcoal can be administered alone or following ipecac or apomorphine-induced emesis. A 10:1 ratio of activated charcoal to acetaminophen reduced absorption by 51% within 30 min (3).

Large quantities of activated charcoal can be administered safely, are well tolerated, and can be used in an emergency for various poisonings (4). To understand the effects of time of administration, pH of the medium, and amount of activated charcoal on the adsorption of acetaminophen, the following study was performed.

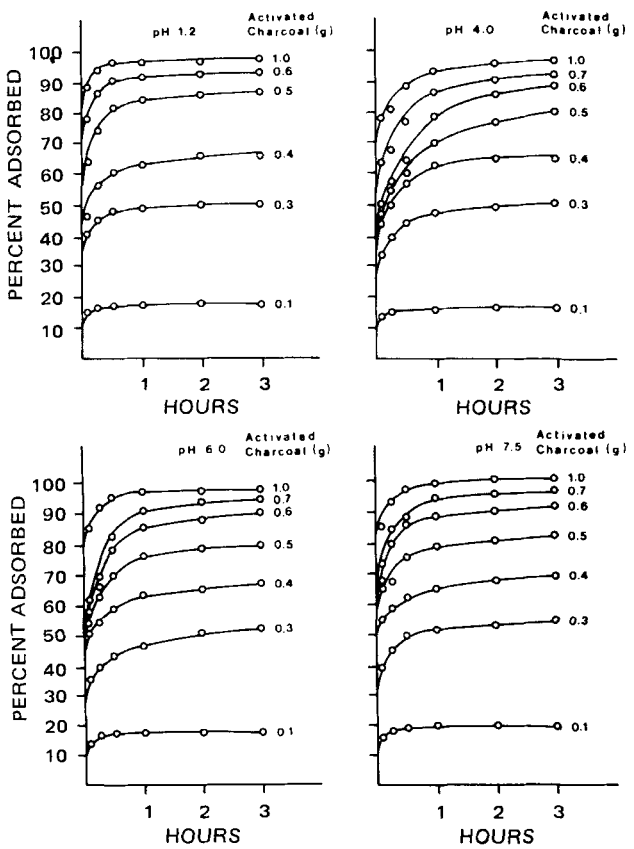

Figure 1—*Percent acetaminophen adsorbed onto activated charcoal at 37.5°.*

Table II—Parameters for the Langmuir Adsorption Isotherm at 37.5°

pH	*n*	*b*	Correlation Coefficient (*r*)
1.2	4.39	0.268	0.996
4.0	4.63	0.223	0.999
6.0	4.16	0.269	0.997
7.5	3.84	0.288	0.998
Composite	4.28	0.282	0.991

EXPERIMENTAL

Materials—Acetaminophen solutions, 2.5 mg/ml, were prepared with the following vehicles: simulated gastric fluid USP without pepsin (pH 1.2), McIlvaine citrate-phosphate buffers (pH 4.0, 4.5, and 6.0), and simulated intestinal fluid USP without pancreatin (pH 7.5).

Analytical—Concentrations of acetaminophen were determined by diluting the samples with acidified methanol and analyzing on a spectrophotometer[1] at 247 nm.

Particle Size—Particle diameters were determined on an automated counter[2].

Adsorption—Various amounts of activated charcoal powder[3] (0.1–1.0 g) were shaken with 50 ml of standard acetaminophen solution at various hydrogen-ion concentrations. The suspensions were shaken at 37.5°, and samples were priodically removed, filtered, and assayed. Samples for equilibrium studies were shaken for 24 hr and centrifuged, and the supernates were assayed.

Desorption—Three variations in desorption experiments were performed.

1. To determine the rate of desorption, various amounts of activated charcoal (0.1–1.0 g) were equilibrated (shaken) overnight with acetaminophen solutions (pH 1.2, 4.5, and 7.5) at 37.5°. The suspensions were centrifuged, the supernates were assayed, and the excess supernates were aspirated. Fifty milliliters of fresh buffer (without acetaminophen) of the same pH as the equilibrating solution was added to the bottles, and the suspensions were shaken again. Samples were removed periodically, filtered, and assayed.

Once it was established that desorption equilibrium could be achieved within 1 hr, the effects of activated charcoal concentration and pH were studied by using only the results from 1-hr samples.

2. Various amounts of activated charcoal (0.2–1.0 g) were equilibrated for 1 hr with pH 1.2 acetaminophen solution. The desorption procedure was the same as Variation 1, but the eluting solutions were of a different pH (4.0 and 7.5).

3. Various amounts of activated charcoal (0.05–1.00 g) were equilibrated for 1 hr with pH 1.2 acetaminophen solution. The desorption procedure was the same as Variation 1 with pH 1.2 eluting solutions. However, each elution was performed four times to determine how repeated elution would affect the accumulative amount of acetaminophen desorbed.

RESULTS AND DISCUSSION

Activated charcoal was demonstrated to be an effective adsorbant for acetaminophen. Within 30 min, most acetaminophen was adsorbed onto the activated charcoal (Fig. 1). Within 1 hr, adsorption was complete at all activated charcoal quantities and over the pH range tested. The percent of acetaminophen adsorbed depended upon the quantity of activated charcoal present. The addition of each 0.1 g of activated charcoal above 0.5 g did not appreciably improve the effectiveness of the activated charcoal for adsorbing 125 mg of acetaminophen. With the reduction of each 0.1 g of activated charcoal below 0.5 g, a large difference in the effectiveness of adsorbing 125 mg of acetaminophen was seen. Consequently, at least four times as much activated charcoal as acetaminophen was required for effective adsorption.

This observation agreed well with suggested doses derived from *in vivo* experiences. Picchioni (5) suggested that a dose of activated charcoal five times the estimated overdose of acetaminophen be employed in acute situations. Levy and Gwilt (3) suggested a 10:1 ratio of activated charcoal to acetaminophen.

[1] Acta III, Beckman Instruments, Fullerton, Calif.
[2] Coulter counter model T, Coulter Electronics Inc., Hialiah, Fla.
[3] Norit A, Matheson, Coleman and Bell, Norwood, Ohio.

Table III—Percent Acetaminophen Adsorbed on Activated Charcoal as a Function of pH at 37.5°

Activated Charcoal, g[a]	Percent Adsorbed			
	pH 1.2	pH 4.0	pH 6.0	pH 7.5
0.05	10.3	8.4	11.2	8.7
0.1	16.8	17.2	19.4	16.4
0.2	29.0	34.1	36.8	36.7
0.3	58.2	50.2	53.7	59.1
0.4	64.9	65.3	68.0	71.0
0.5	77.7	77.7	81.2	85.3
0.6	87.2	87.0	90.0	93.9
0.7	94.5	89.9	95.9	97.2
1.0	98.3	98.8	99.0	99.7

[a] Quantities per 50 ml of acetaminophen solution, 2.5 mg/ml.

Activated charcoal powder from another source[4] was compared to the activated charcoal[3] employed throughout the rest of the study. The mean particle diameters of the activated charcoal powder from the two sources were similar (one source[4] had a mean of 8.3 μm and a 70% range of 3.6–16.5 μm; the other[3] had a mean of 9.1 μm and a 70% range of 3.7–18.5 μm). Only slight differences in their equilibrium concentrations were found. Table I shows the differences in the percent adsorbed at equilibrium onto the two brands of activated charcoal. These differences should not have a bearing on the effectiveness of activated charcoal in overdose situations.

Langmuir adsorption isotherms were determined for the adsorption of acetaminophen onto activated charcoal powder at various hydrogen-ion concentrations. The Langmuir isotherm is defined as follows:

$$c/(x/m) = nc + b \qquad \text{(Eq. 1)}$$

where c = concentration of acetaminophen in milligrams per milliliter at equilibrium, x/m = amount of adsorbed drug in milligrams (x) per milligram of activated charcoal (m), and n and b are constants for the isotherms. The constants (n and b) were determined by linear regression analysis at each pH (Table II). Since the differences between the isotherms at various pH values were not significant, the data from all four pH values were pooled and a composite isotherm was obtained. The parameters for this isotherm are listed in Table II as "composite."

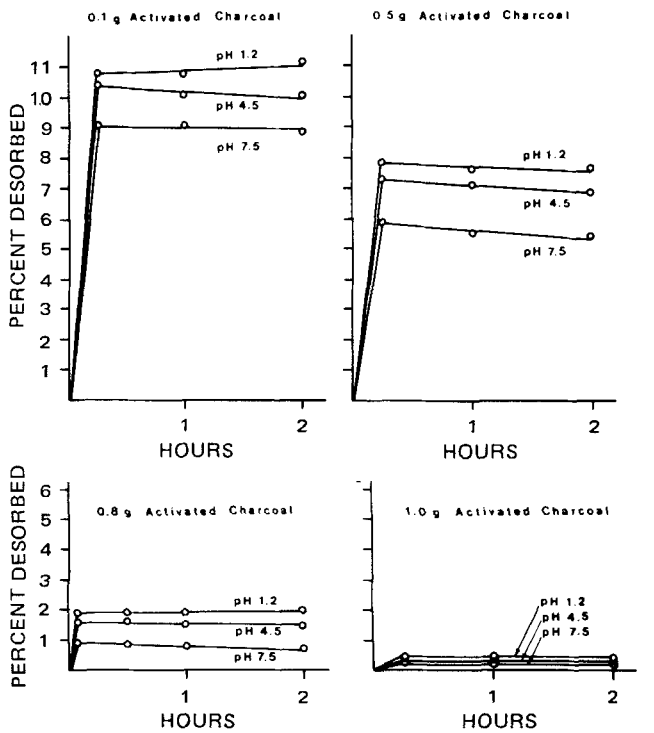

Figure 2—*Percent acetaminophen desorbed from activated charcoal at 37.5°.*

[4] Amend Drug and Chemical Co., New York, N.Y.

Table IV—Percent Acetaminophen Desorbed from Activated Charcoal at 37.5°

Activated Charcoal, g[a]	Percent Desorbed	
	pH 4.0	pH 7.5
0.2	25.7	17.8
0.3	19.9	13.7
0.4	15.9	10.2
0.5	12.0	7.2
0.6	8.3	4.4
0.7	5.8	2.3
1.0	1.4	0.3

[a] Equilibrium was attained with 50 ml of pH 1.2 acetaminophen solution, 2.5 mg/ml, and elution was performed with 50 ml of pH 4.0 and 7.5 buffers.

Table V—Desorption by Four Successive Elutions at 37.5°[a]

Activated Charcoal, g	Accumulative Percent Desorbed			
	Elution 1	Elution 2	Elution 3	Elution 4
0.05	30.8	40.3	45.8	48.8
0.1	26.5	36.1	41.4	45.1
0.2	18.9	27.5	32.5	36.0
0.3	13.7	21.0	25.7	29.0
0.4	10.4	16.5	20.6	23.6
0.5	7.4	12.2	15.7	18.8
0.6	4.2	7.3	9.9	12.0
0.7	2.0	3.7	5.1	6.5
1.0	0.4	0.7	0.9	1.2

[a] Equilibrium was attained with 50 ml of pH 1.2 acetaminophen solution, 2.5 mg/ml, and elution was performed with 50 ml of pH 1.2 solution.

Adsorption onto activated charcoal is greater for unionized compounds. Since acetaminophen has a pKa of 9.9 (6), it is unionized in the pH 1.2–7.5 range. Therefore, the compound is well adsorbed at all physiological pH values. Under *in vivo* conditions, small differences such as were seen in the Langmuir isotherms would probably be negated by ions, enzymes, *etc.*, which would also be adsorbed onto the charcoal.

The maximum adsorption capacity (1/n) was 234 mg of acetaminophen/g of activated charcoal. This value is in agreement with the 279 mg of acetaminophen/g of activated charcoal found by Levy and Gwilt (3).

The effect of pH on the percent of acetaminophen adsorbed at equilibrium onto activated charcoal is more evident from the data in Table III. For each activated charcoal quantity, only a slight difference was seen. For example, 0.5 g of activated charcoal adsorbed 77.7% of acetaminophen at pH 1.2 and 85.3% at pH 7.5. Normal deviations in data and the use of different buffer systems might account for the differences seen.

Desorption studies were performed to determine if acetaminophen, once adsorbed, could be desorbed from activated charcoal as a result of a change in pH or in the acetaminophen concentration in the solution. Figure 2 illustrates the effect of variations in the quantity of activated charcoal employed and in the pH of the desorption medium. Desorption was essentially complete within 15 min. Desorption decreased with decreasing pH and with increasing quantities of activated charcoal.

Table IV describes desorption when the eluting solution was at a pH (4.0 and 7.0) higher than the pH (1.2) of the adsorbing solution. Desorption was greater in the pH 4.0 solution than in the pH 7.0 solution. The exact reason for this result is not known, but it is probably related to the buffer compositions. Also, as in the previous case, the greater the amount of activated charcoal employed, the smaller was the percent desorption of acetaminophen.

Data in Table V indicate that the most desorption occurred with the first elution. Subsequent elutions had a much smaller effect on desorption, especially when large amounts of activated charcoal were employed.

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* Present address: Medical College of Pennsylvania, Philadelphia, PA 19129.

‡ Present address: Villanova University, Villanova, PA 19085.

x To whom inquiries should be directed.

Conformational Studies of Antiradiation Agents by NMR: Cysteamine and Its Derivatives

VITHAL M. KULKARNI and GIRJESH GOVIL x

Abstract □ The conformations of cysteamine, thiazolidine, and thiazolidine-4-carboxylic acid were determined in aqueous solutions using NMR spectroscopy. At physiological pH, the population ratio of *gauche*- and *trans*-conformers was 3:1. The *gauche*-rotamer is probably responsible for the antiradiation activity and acts through metal chelation involving sulfur and nitrogen atoms. The puckering of the thiazolidine ring was calculated using NMR coupling constants. The observed results were compared with those obtained in the solid state using X-ray diffraction.

Keyphrases □ Cysteamine—and derivatives, NMR conformational study in aqueous solutions □ Thiazolidine—and derivatives, NMR conformational study in aqueous solutions □ NMR—conformational study of cysteamine, thiazolidine, and derivatives in aqueous solutions □ Conformations—cysteamine, thiazolidine, and derivatives, NMR study in aqueous solutions □ Antiradiation agents—cysteamine, thiazolidine, and derivatives, NMR conformational study in aqueous solutions

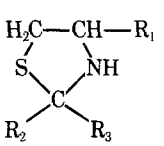
Cysteamine (2-aminoethanethiol, I) is a well-established antiradiation agent (1). The sulfur-substituted derivative of this molecule (2), 2-aminoethanethiosulfuric acid (II), is as active as I. Some 2-substituted thiazolidines (III and IV), prepared by condensing I with carbonyls, are also good antiradiation agents (3). To explain the mechanism of radioprotection offered by these compounds, several postulations (4, 5) have been suggested such as mixed disulfide formation (6) with protein constituents, chelation of vital metal ions (7), and binding to DNA (8).

The conformations in the solid state by X-ray diffraction for these molecules were reported (9–12). Recently, quantum chemical calculations using extended Hückel theory (EHT) and complete neglect of differential overlap (CNDO) methods were made in this laboratory. The results showed that I has both *gauche*- and *trans*-conformations, with a preference toward the *trans*-structure.

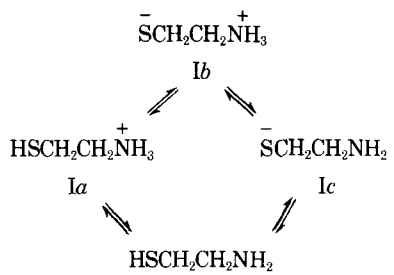
In this paper, the application of NMR spectroscopy for the determination of the preferred conformation in solution of cysteamine and some thiazolidine derivatives is reported. Attempts were made to correlate these findings with their biological action.

$HSCH_2CH_2NH_2$ I

$HO_3SSCH_2CH_2NH_2$ II



III: $R_1 = COOH$, $R_2 = R_3 = H$
IV: $R_1 = H$, $R_2 = R_3 = CH_3$



Scheme I

In physiological solution, cysteamine undergoes multiple ionization (9) (Scheme I). The ionization constants governing these equilibria have been reported, so it is possible to calculate the fraction of each species as a function of pH. The concentration of I is low at all pH values. Form I*a* predominates in acidic media, while I*c* predominates at pH values greater than 12. Form I*b* is present at intermediate pH's and reaches its maximum concentration of 84.6% around pH 9.5. Therefore, NMR studies of cysteamine were made at three different pH values. Other compounds were studied in neutral solution and in deuterochloroform.

EXPERIMENTAL

Materials—Cysteamine hydrochloride[1] was recrystallized from 95% ethyl alcohol until it was free from disulfide traces. 2-Aminoethanethiosulfuric acid and thiazolidine derivatives were prepared using reported procedures (3, 13).

NMR Measurements—Samples were dissolved in deuterium oxide to a known concentration, and spectra[2] were recorded using the sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid as the internal reference. The pH values were adjusted using trifluoroacetic acid.

RESULTS AND DISCUSSION

Spectral Analysis—The studied molecules are of the 1,2-disubstituted ethane type and show the usual rotational isomerism around the carbon–carbon bond. In the case of thiazolidines, this rotation is hindered, but a small degree of conformational freedom is still present due to the puckering modes of the ring. The spectrum of cysteamine recorded in

[1] Fluka grade.
[2] Varian Associates HA-100 and A-60 spectrometers.