# Determination of Pentobarbital in Serum by Electron-Capture GLC

**SY-RONG SUN** [x] **and A. H. C. CHUN**

**Abstract** □ A GLC method was developed for pentobarbital in serum. After extraction from serum, a pentafluorobenzyl derivative was prepared and quantitated by electron-capture detection. The method has a sensitivity of 0.1 μg/ml of serum, and the amount detectable is less than 0.2 ng/injection. Hexethal was used as the internal standard. Derivatives of other barbiturates were also made. NMR and mass spectrometric analyses confirmed the proposed structure of the 1,3-bis(pentafluorobenzyl) derivative of pentobarbital. The procedure was successfully applied to measurement of serum pentobarbital levels in humans.

**Keyphrases** □ Pentobarbital—electron-capture GLC analysis, human serum □ GLC, electron capture—analysis, pentobarbital in human serum, retention times, various barbiturates □ Barbiturates, various—electron-capture GLC retention times □ Sedatives—pentobarbital, electron-capture GLC analysis in human serum, retention times, various barbiturates

The GLC determination of barbiturates was reported by numerous authors in the past several years. Some investigators (1, 2) methylated the barbiturate with dimethyl sulfate prior to GLC analysis. A direct "on-column" flash-heater methylation of barbiturates with trimethylanilinium hydroxide was also reported. Ehrnebo *et al.* (4) used similar methodology to assay amobarbital and pentobarbital in serum after ether extraction at pH 5.5. Other investigators (5–12) also described the determination of barbiturates in biological fluids. These methods primarily utilized GLC with flame-ionization detection.

This report describes a new GLC method for the determination of pentobarbital (I). It is sensitive to 0.2 ng/injection, using electron-capture detection of the 1,3-bis(pentafluorobenzyl) derivative of pentobarbital (III, Scheme I) in serum.

## EXPERIMENTAL

**Reagents and Materials**—Pentobarbital[1] (I), hexethal[2] (II), the internal standard, and pentafluorobenzyl bromide[3] were used as supplied. Ether[4] and benzene[4] were nanograde, absolute alcohol[5] was USP grade, and the other chemicals were analytical reagent grade. Aqueous solutions of sodium tungstate (10%), sodium bicarbonate (3%), sodium carbonate (250 μg/ml), and sulfuric acid (1 *N*) were stored in glass containers.

**Apparatus**—A two-speed reciprocating shaker[6] and a refrigerated centrifuge[7] were used. Sample solutions in the test tubes were mixed by a mixer[8]. Pentafluorobenzyl derivative formation at high temperature was carried out on a heating block[9].

**GLC**—A gas chromatograph[10] equipped with a $^{63}$Ni-electron-capture detector containing a 2-mCi $^{63}$Ni-β-ionization source and an electronic integrator were used for the GLC assay. The glass column was 1.82 m (6 ft) × 2 mm (i.d.), packed with 3% OV-17[11] on Gas Chrom Q[11] (80–100 mesh). The column was conditioned at 280° for 1 hr without carrier gas flow and for 16 hr with a carrier gas [argon–methane[12] (95:5)] at a flow rate of 50 ml/min. Before connecting the column to the detector, three to five 5-μl injections of *N,O*-bis(trimethylsilyl)acetamide[13] were made into the column every 10 min.

The cylinder of the carrier gas was fitted with an oxygen trap filter[14]. The operating conditions were: column oven temperature, 225°; electron-capture detector temperature, 325°; injection port temperature,

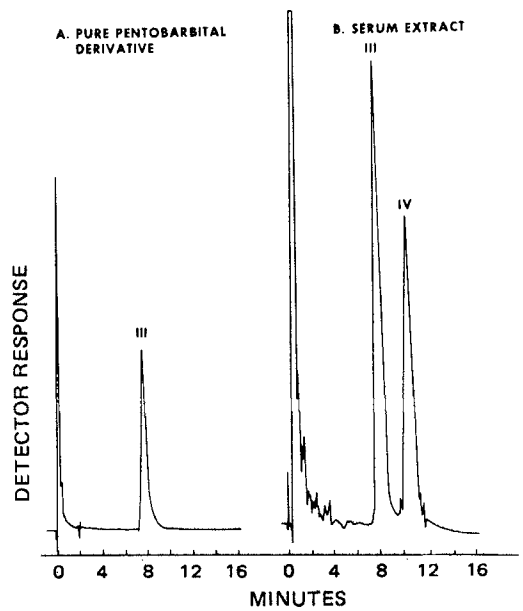



**Figure 1**—*Gas-liquid chromatogram (electron-capture detection) of pentafluorobenzyl derivatives. Key: A, 0.2 ng of pure III injected; B, serum extract from human subject at 24 hr after single-dose oral administration of 100 mg of pentobarbital sodium (capsule form); and IV, pentafluorobenzyl derivative of the internal standard (hexethal).*

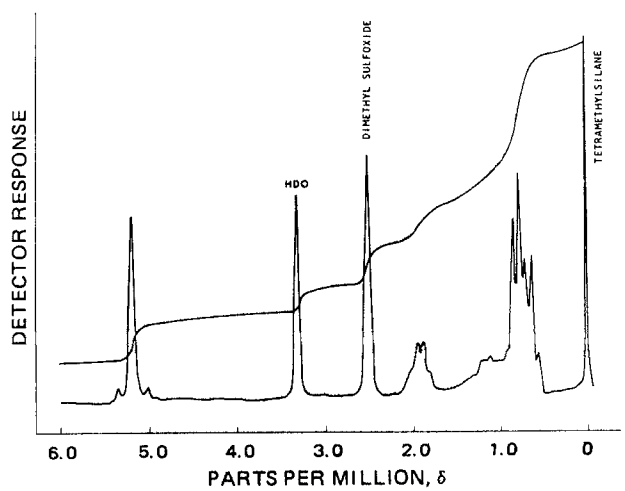



**Figure 2**—*NMR spectrum of III.*

[1] Internal reference standard, Nembutal acid, lot 3330-113, Abbott Laboratories, North Chicago, Ill.
[2] Internal reference standard, lot A-13271, Abbott Laboratories, North Chicago, Ill.
[3] Bendix Corp., Ronceverte, W. Va.
[4] Mallinckrodt Chemicals, St. Louis, Mo.
[5] Reagent, Abbott Laboratories, North Chicago, Ill.
[6] Eberbach Corp., Ann Arbor, Mich.
[7] Model RC-3, Sorvall, Newtown, Conn.
[8] Vortex Genie model K-550-GT, Scientific Industries, Springfield, Mass.
[9] Dri-block DB-3, Techne, Inc., Princeton, N.J.
[10] Hewlett-Packard model 7620.
[11] Applied Science Laboratories, State College, Pa.
[12] Matheson Gas Products, Elk Grove Village, Ill.
[13] Pierce Chemicals Co., Rockford, Ill.
[14] Altech Associates, Arlington Heights, Ill.

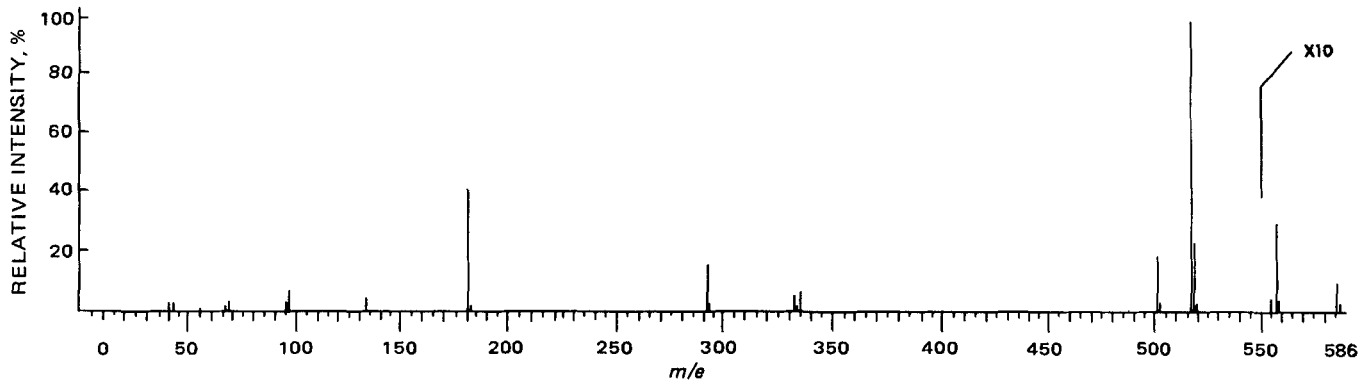
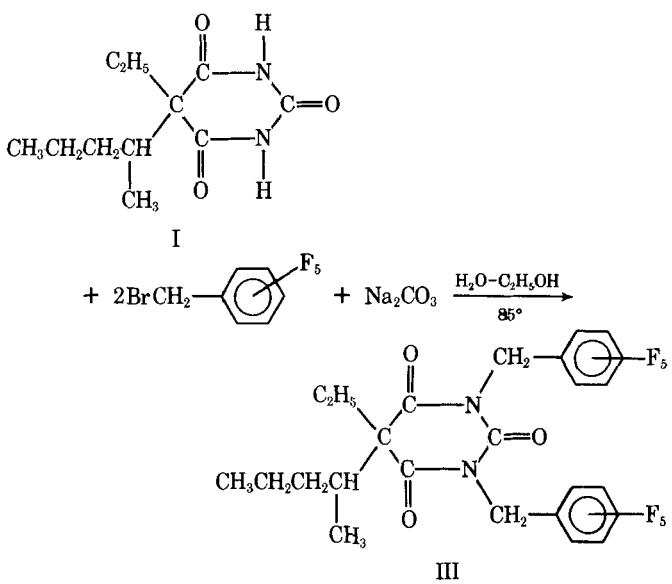


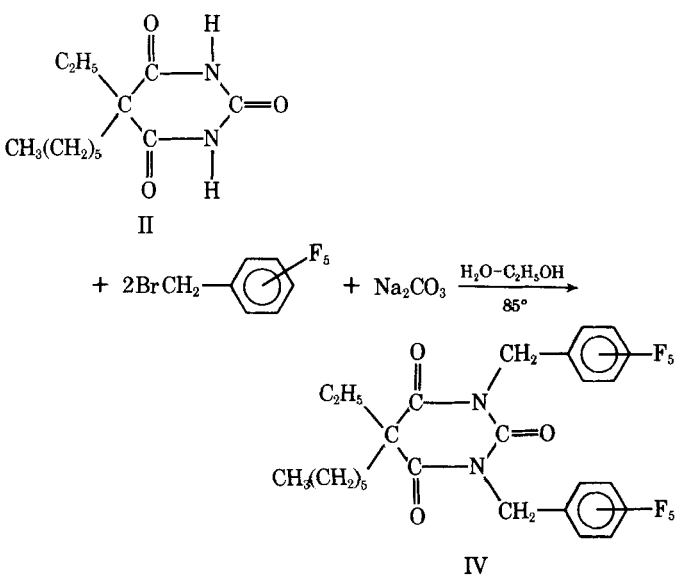

**Figure 3**—*Mass spectrum of III.*

250°; carrier gas flow rate, 50 ml/min; detector pulse interval, 150 μsec; electrometer range, $10^3$; recorder presentation, 2 mv; and slope sensitivity, 0.3 mv/min.

**Spectrometry**—A mass spectrometer[15] was used under the following conditions: energy of the ionization beam, 70 ev; ion source temperature,




*Scheme I*




*Scheme II*

[15] MS-902, Associate Electronic Industry, Manchester, England.

160°; accelerating voltage, 8 kv; and trap current, 100 μamp. The NMR spectrum was recorded at ambient temperatures on a spectrometer[16] using a solution in dimethyl sulfoxide containing tetramethylsilane as the internal standard. The IR spectrum[17] was measured using a potassium bromide pellet.

**Preparation of Serum Standards**—For human studies, serum standards spiked with I at 0.5, 1.0, 1.5, 2.0, and 2.5 μg/ml were used. This range covered the human serum pentobarbital levels expected after oral administration of a 100-mg dose of pentobarbital sodium capsules[18].

**Extraction Procedure**—To 1.0 ml of serum in a 20-ml screw-capped test tube were added 3.0 ml of hexethal internal standard solution in water (1 μg/ml) and 1.0 ml of 1 *N* $H_2SO_4$. The tube was vortexed, 1.0 ml of 10% sodium tungstate solution in water was added, and the tube was then shaken mechanically for 10 min. After centrifugation at 3000 rpm for 15 min at 20°, all of the aqueous phase (supernate) was separated and transferred to a 15-ml conical test tube and recentrifuged at 3000 rpm for 10 min at 20°. Four milliliters of aqueous phase was then pipetted into a 20-ml screw-capped test tube and extracted with 11.5 ml of ether by shaking for 10 min and centrifuging at 3000 rpm for 10 min at 10°.

Ten milliliters of the organic phase was transferred to a 20-ml screw-capped test tube and washed with 2 ml of 3% $NaHCO_3$ aqueous solution by shaking and centrifuging as previously described. The ether layer (9 ml) was transferred into the 15-ml screw-capped conical test tube and evaporated to dryness at 40° with filtered air. The residue was reconstituted with 1 ml of ether and dried again at 40° with filtered air.

**1,3-Bis(pentafluorobenzyl) Derivative (III) Formation**—To the dry residue were added 0.1 ml of sodium carbonate solution (250 μg/ml of water), 0.1 ml of alcohol, and 50 μl of pentafluorobenzyl bromide in alcohol (2 μg/μl) (Schemes I and II). The mixture was capped and vortexed twice for 15 sec and reacted at 85° for 4 hr on the heating block. Then the mixture was cooled to room temperature; 0.5 ml of water and 4.5 ml of benzene were added, followed by shaking and centrifugation for 10 min. Four milliliters of the benzene extract was separated and evaporated to dryness at 40° with filtered air. The clear residue was reconstituted with 1.0 ml of benzene by vortexing for about 30 sec, and 2 μl of the solution was injected into the GLC column by the automatic sampler[19].

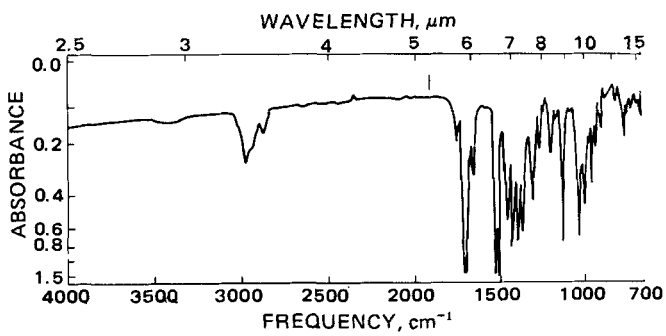



**Figure 4**—*IR spectrum of III in potassium bromide pellet.*

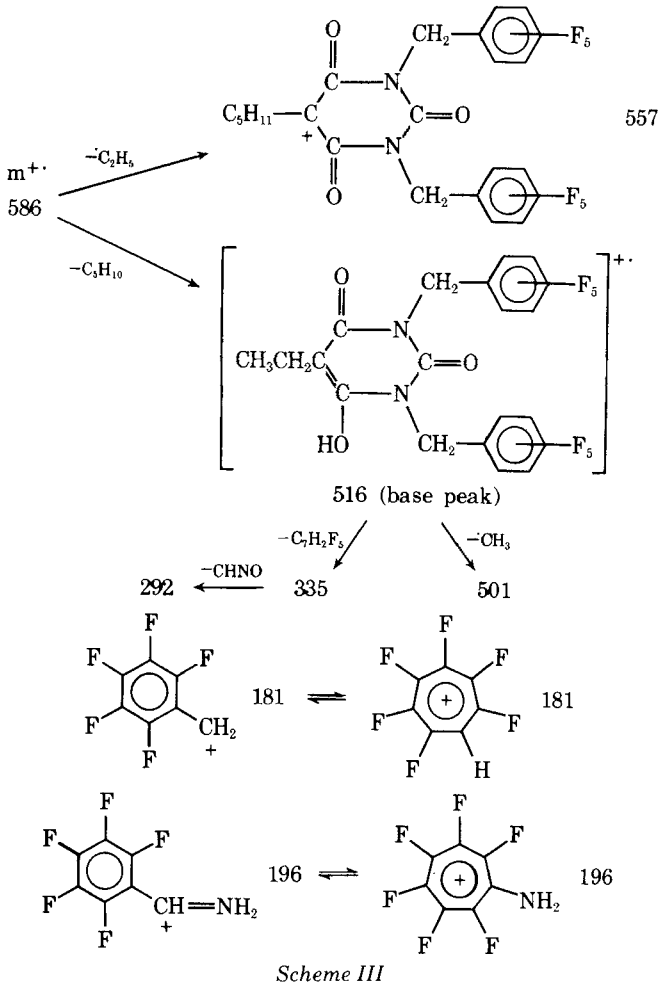
[16] Varian XL-100.
[17] Perkin-Elmer model 521 IR spectrometer.
[18] Nembutal sodium capsules, 100 mg, lot 38-465-AR, Abbott Laboratories.
[19] Hewlett-Packard model 7670A.

Table I—Peak Area Ratio of III to IV at Various Pentobarbital Serum Concentrations

| Pentobarbital Serum Concentration, μg/ml | Peak Area Ratio | Mean ± *SD* | *RSD* |
|---|---|---|---|
| 0.1 | 0.35<br>0.32<br>0.38 | 0.35 ± 0.03 | 8.6 |
| 0.2 | 0.83<br>0.70<br>0.73 | 0.75 ± 0.07 | 9.3 |
| 0.3 | 1.03<br>1.39<br>1.40 | 1.27 ± 0.21 | 16.5 |
| 0.4 | 1.70<br>1.82<br>2.14 | 1.89 ± 0.23 | 12.2 |
| 0.5 | 2.25<br>2.39<br>2.21 | 2.28 ± 0.10 | 4.4 |
| 0.6 | 2.54<br>2.73<br>2.70 | 2.66 ± 0.10 | 3.8 |
| 0.8 | 3.64<br>3.69 | Average 3.67 | — |
| 1.0 | 4.92<br>5.17<br>5.13 | 5.07 ± 0.13 | 2.6 |

Under these conditions, derivatization of pentobarbital with pentafluorobenzyl bromide gave one peak on GLC analysis with a retention time of 7.7 min. Hexethal also gave a single peak with a retention time of 10.6 min. Spiked standards of pentobarbital in serum were extracted and reacted with pentafluorobenzyl bromide as previously described.

**Calculations**—The peak area ratios of III to IV were plotted against known standards of pentobarbital expressed as micrograms per milliliter of serum. Values for unknown concentrations of pentobarbital in serum were calculated by the least-squares regression method from the calibration curve.




*Scheme III*

## RESULTS AND DISCUSSION

**Synthesis and Identification of 1,3-Bis(pentafluorobenzyl) Derivative of Pentobarbital (III)**—Structural confirmation of III was accomplished by synthesizing sufficient pure drug under similar conditions. The derivative collected was white crystalline material, mp 106–107°. The gas chromatogram (Fig. 1A) of pure III thus formed was identical to that prepared with the serum extract under the same conditions (Fig. 1B).

The NMR spectrum (Fig. 2) showed the presence of benzyl protons at a chemical shift 5.20 ppm downfield from tetramethylsilane. High-resolution mass spectral measurement (Fig. 3) confirmed a small, but distinct, molecular ion at *m/e* 586, consistent with the proposed structure (III). From the empirical formulas calculated, Scheme III was proposed, indicating two pentafluorobenzyl groups attached to the two nitrogen atoms. The IR spectrum (Fig. 4) showed bands at 1700 and 1760 $cm^{-1}$, assigned to carbonyl stretching. This spectrum also indicates that derivatization occurs on the ring nitrogens and not on the oxygen.

**Assay Sensitivity, Reproducibility, and Specificity**—The amount of III detectable by the electron-capture detector was less than 0.2 ng/

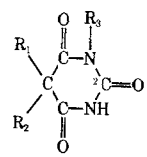


Table II—Retention Times of the Pentafluorobenzyl Derivatives of 15-Barbiturates by the Electron-Capture Detector

| Barbiturate | $R_1$ | $R_2$ | $R_3$ | $R_f$, min |
|---|---|---|---|---|
| Metharbital[a] | $C_2H_5$ | $C_2H_5$ | $CH_3$ | 1.34 |
| Phenobarbital | $C_2H_5$ | $C_6H_5$ | H | 1.83 dec. |
| Barbital | $C_2H_5$ | $C_2H_5$ | H | 4.73 |
| Hexobarbital | $CH_3$ | $C_6H_9$ | $CH_3$ | 5.15 |
| Allobarbital | $CH_2$=$CHCH_2$ | $CH_2$=$CHCH_2$ | H | 5.66 |
| Mephobarbital | $C_2H_5$ | $C_6H_5$ | $CH_3$ | 5.66 |
| Butalbital | $CH_2$=$CHCH_2$ | $(CH_3)_2CHCH_2$ | H | 5.76 |
| Aprobarbital | $CH_2$=$CHCH_2$ | $(CH_3)_2CH$ | H | 6.08 dec. |
| Butethal | $C_2H_5$ | $C_4H_9$ | H | 6.35 |
| Butabarbital | $C_2H_5$ | $CH_3CH_2CH(CH_3)$ | H | 6.59 dec. |
| Amobarbital | $C_2H_5$ | $(CH_3)_2CHCH_2CH_2$ | H | 6.85 dec. |
| Pentobarbital | $C_2H_5$ | $CH_3CH_2CH_2CH(CH_3)$ | H | 7.71 |
| Thiopental (S replaces O on C-2) | $C_2H_5$ | $CH_3CH_2CH_2CH(CH_3)$ | H | 7.86 |
| Secobarbital | $CH_2$=$CHCH_2$ | $CH_3CH_2CH_2CH(CH_3)$ | H | 8.39 |
| Hexethal | $C_2H_5$ | $CH_3(CH_2)_5$ | H | 10.58 |

[a] Gemonil, Abbott.

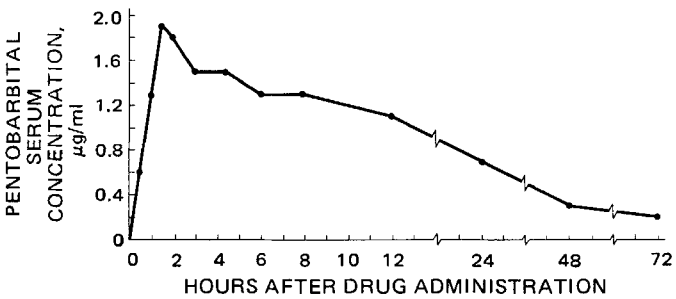



**Figure 5**—*Serum concentration of pentobarbital* versus *time in one human subject after single-dose oral administration of 100 mg of drug/capsule.*

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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# *In Vitro* Adsorption of Acetaminophen onto Activated Charcoal

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**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