difference centers around the increased prominence and number of hair follicles. Probably diffusion across the transfollicular shunt is greatly enhanced, to the point where it becomes the principal pathway. The horny layer of the epidermis is also affected by the formation of a thick coat of hair. Since the hairy mat restricts insensible perspiration, the stratum corneum associated with furry skin need not be as impermeable to water, and it is typically thinner and less well formed than when the surface is hairless. Thus, structural and compositional changes in the horny layer also provide a basis for explaining the differing hydration sensitivities. It is likely that both factors are important.

This study provides further evidence of intra- and interspecies complexities of animal skins as mass transport regulators. To a degree it demonstrates that skins covered with thick coats of hair do not behave diffusionally as hairless skins, whether of mouse or humans. Previous work (1–8) indicates a high degree of parallelism in the chemical barrier properties of hairless mouse and human skins. This study adds support for the use of hairless animals for research on percutaneous absorption, primary irritancy, topical drug delivery, *etc.*, when it is not possible or practicable to use human subjects.

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NOTES

In Vitro Adsorption of Phenobarbital onto Activated Charcoal

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Abstract ☐ *In vitro* experiments were performed to determine the extent and duration of adsorption and desorption of phenobarbital onto and from activated charcoal in solutions of various pHs. The results of studies supported the evidence of the effectiveness of charcoal as an adsorbent. Adsorption was dependent upon the quantity of charcoal used. With amounts of charcoal ≥0.5 g, adsorption was complete within 60 min. Desorption was rapid, quantity dependent, and pH independent. The results of adsorption isotherms indicated no change in binding capacity of the drug from solutions of different pH.

Keyphrases ☐ Phenobarbital—*in vitro* adsorption onto activated charcoal, desorption, binding ☐ Adsorption, *in vitro*—phenobarbital onto activated charcoal, desorption, binding

The importance of activated charcoal cannot be overemphasized as an emergency treatment in drug poisoning. Activated charcoal given orally as a slurry can effectively adsorb and hold many drugs such as alkaloids, glycosides, and barbiturates (1). Acute barbiturate poisoning is common and it accounts for ~1500 deaths annually in the United States (2). Barbiturates are the second most frequent cause of poisoning in children (3).

Phenobarbital is one of the major barbiturates used in many products as a sedative–hypnotic and antiepileptic agent. It has a therapeutic range of 0.03–0.6 g daily in divided doses, and its potential for poisoning is great, having a fatal range of 1–10 g (4). Data collected on the use of phenobarbital in adsorption studies with activated charcoal can be useful.

A slurry of activated charcoal given 30 min after hypnotic doses of phenobarbital or glutethimide resulted in a plasma drug concentration at least 50% lower in treated than in untreated dogs, even when charcoal was allowed to pass through the entire GI tract (5). Adsorbed material is retained tenaciously throughout passage in the gut. There is a concern that part of the poison in the intestine may later be released because of less favorable pH conditions. It was found that the reduction in the amount of available poison is markedly high as compared to insignificant elution of the poison in the intestine (6). Administration of large amounts of activated charcoal is considered to be a routinely useful procedure. In view of the delayed emetic action of ipecac syrup, there is an increasing speculation that activated charcoal may be of more importance as an emergency treatment for accidental poisoning. Recent studies (7–9) have shown the importance of adsorption of different drugs onto activated charcoal.

The purpose of this study was to investigate and understand the extent of adsorption of phenobarbital sodium

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Table I—Percent of Phenobarbital Desorbed at the End of 2 hr from Various Concentrations of Activated Charcoal [a] by Solutions of Various pH

pH	Charcoal Concentration, g			
	0.1	0.5	0.7	1.0
1.2	54.0	4.61	0.88	0.59
4.0	52.3	4.55	1.64	1.69
8.0	51.5	3.87	1.82	0.91

[a] For prior adsorption, different charcoal amounts were equilibrated for 2 hr with standard solution of phenobarbital sodium at pH 1.2.

onto activated charcoal as related to the effect of time, duration of equilibrium, extent of desorption, and the pH of the medium.

EXPERIMENTAL

Materials—Phenobarbital sodium BP[1] solutions, 1 mg/ml, were prepared with the following vehicles: simulated gastric fluid, USP, without pepsin, pH 1.2; McIlvaine's citrate-phosphate buffer, pH 4.0; phosphate buffer USP, pH 6.0; alkaline borate buffer USP, pH 8.0 and 9.6.

Analysis—Concentrations of phenobarbital calculated as phenobarbital sodium were determined by diluting the samples with alkaline borate buffer pH 9.6 and reading them on a spectrophotometer[2] at 240 nm.

Adsorption—Various amounts of activated charcoal[3] (0.1–1.0 g) were shaken for different time intervals, each with 50 ml of the standard phenobarbital sodium solution with pH values of 1.2, 4.0, 6.0, and 8.0. The suspensions were shaken at 44 rpm in a water bath at 37.5°. Samples were periodically removed, filtered through a double filter, and assayed. For adsorption isotherms, suspensions of 50 mg of charcoal were shaken with 50 ml of 0.02, 0.03, 0.05, 0.075, 0.09, and 0.1% solutions of the drug for 24 hr. Suspensions were filtered and the filtrate assayed.

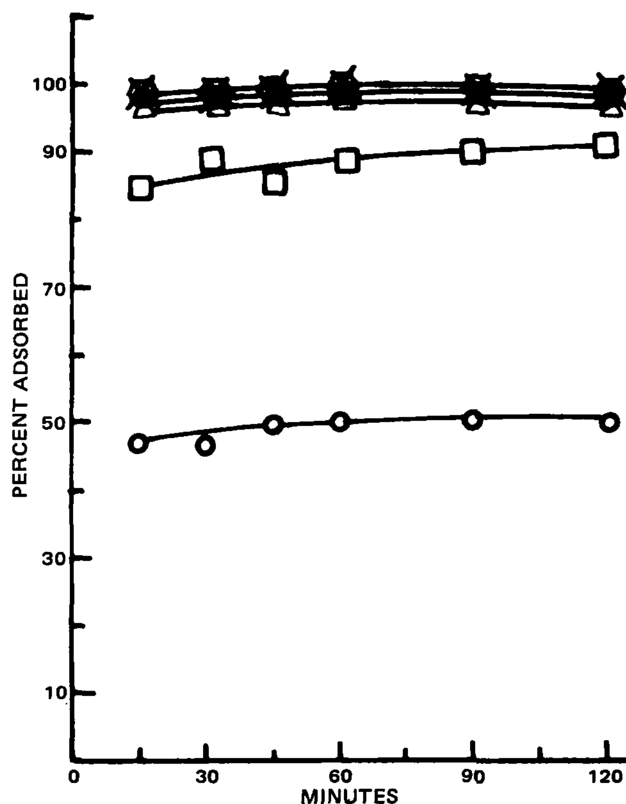

Figure 1—Percent of phenobarbital adsorbed by various concentrations of activated charcoal from a solution of pH 1.2. Key: (○), 0.1 g; (□) 0.3 g; (△) 0.5 g; (●) 0.7 g; (×) 0.9 g; (○) 1.0 g.

[1] Koch-Light Laboratories Ltd., Colnbrook Bucks, England.
[2] Spectrophotometer UV, Model 150-02, Shimadzu Seisakusho Ltd., Kyoto, Japan.
[3] BDH Chemicals Ltd., Poole, England.

Table II—Value of Constants of Langmuir Adsorption Isotherms at 37.5° in Solutions of Various pH

pH	Slope (n)	Adsorption Capacity $\left(\frac{1}{n}\right)$, mg	Intercept (b)	Correlation Coefficient (r)
1.2	3.42	292	0.62	1.00
4.0	3.76	265	0.65	1.00
6.0	3.86	259	0.86	1.00
8.0	3.83	261	1.17	1.00

Desorption—Various amounts of charcoal powder were equilibrated for 2 hr with the standard solution of phenobarbital sodium at pH 1.2. Two hours was selected for equilibrium during adsorption studies. This time period was found to be sufficient for adsorption to be complete. The samples were centrifuged at 2500 rpm; supernatants were aspirated and assayed. Fifty milliliters of fresh buffers without phenobarbital sodium at pH 1.2, 4.0, and 8.0 was added as an eluting medium to transfer the sediments to the flasks. The suspensions were shaken for 15, 30, 60, and 120 min. The samples were periodically removed, filtered, and assayed.

Particle Size—Average particle diameter for the activated charcoal powder used was 5.1 μm. It was determined by an air permeability device[4].

RESULTS AND DISCUSSION

Activated charcoal is a potent adsorbent that rapidly inactivates many poisons if administered before much absorption of poison has taken place. The rapid and marked effectiveness of activated charcoal *in vivo* emphasizes the potential usefulness of this antidote in the management of acute poisoning due to a rapidly absorbed chemical agent. Adsorption

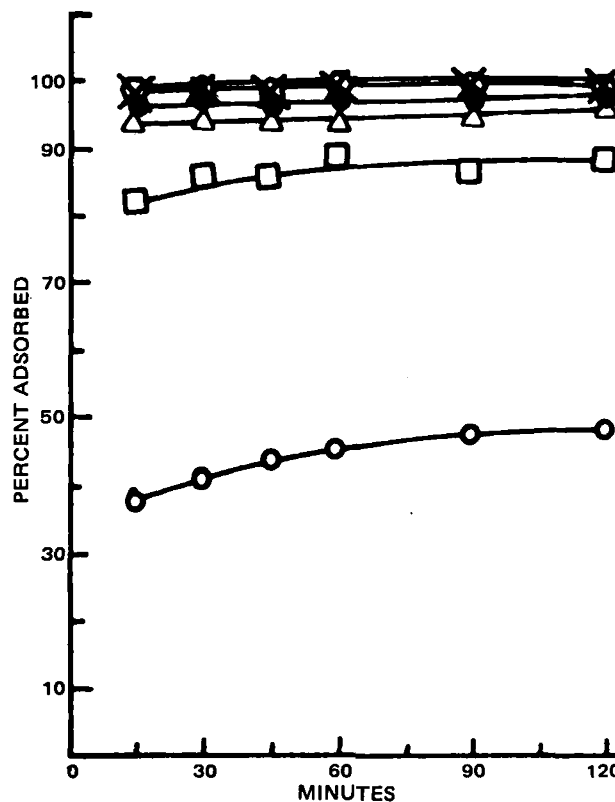

Figure 2—Percent of phenobarbital adsorbed by various concentrations of activated charcoal from a solution of pH 4.0. Key: (○) 0.1 g; (□) 0.3 g; (△) 0.5 g; (●) 0.7 g; (×) 0.9 g; (○) 1.0 g.

[4] Fisher-Sub-Sieve Sizer, Model 95, Fisher Scientific Co., Pittsburg, Pa.

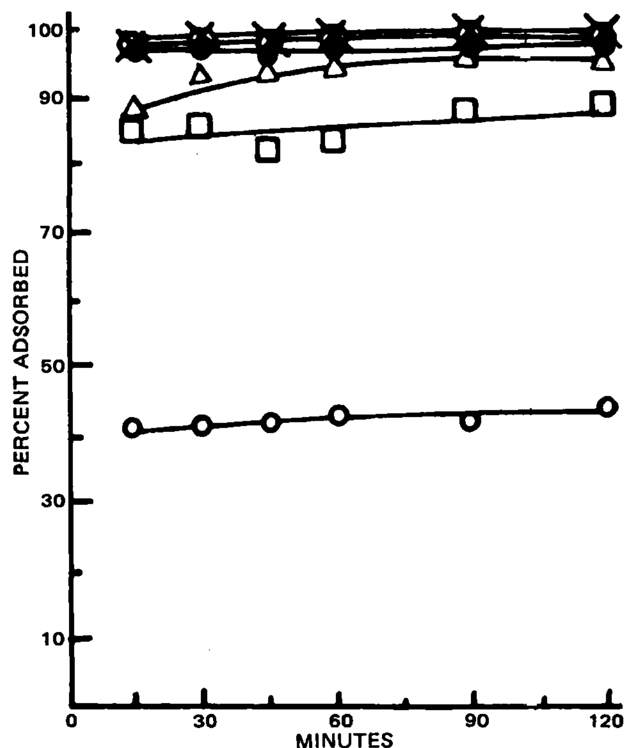

Figure 3—*Percent of phenobarbital adsorbed by various concentrations of activated charcoal from a solution of pH 6.0. Key: (○) 0.1 g; (□) 0.3 g; (△) 0.5 g; (●) 0.7 g; (×) 0.9 g; (○) 1.0 g.*

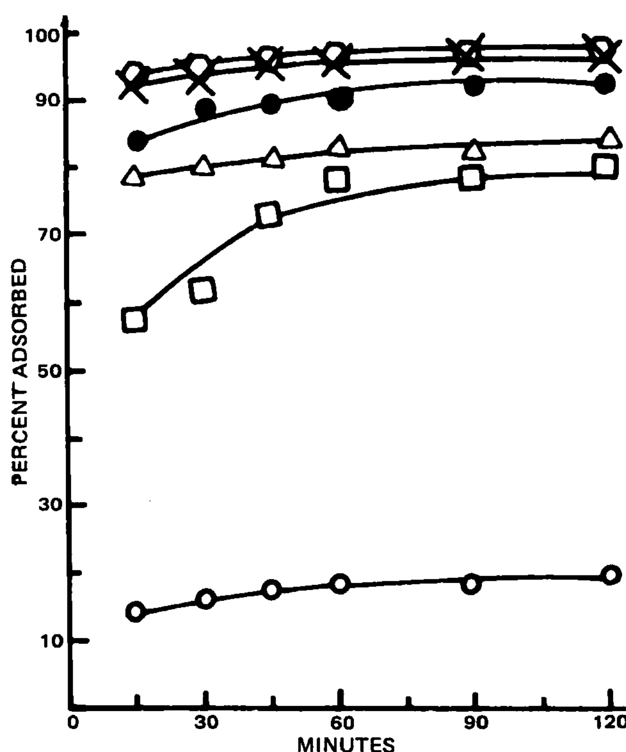

Figure 4—*Percent of phenobarbital adsorbed by various concentrations of activated charcoal from a solution of pH 8.0. Key: (○) 0.1 g; (□) 0.3 g; (△) 0.5 g; (●) 0.7 g; (×) 0.9 g; (○) 1.0 g.*

experiments were performed to see the amounts of phenobarbital adsorbed onto activated charcoal as a function of time and hydrogen ion concentration.

Figures 1–3 show that an increase in the amount of activated charcoal resulted in higher amounts of phenobarbital adsorbed from solutions of pH 1.2, 4.0, and 6.0. A sudden rise was noted when the amount of charcoal was increased from 0.1 to 0.3 g. In solutions of pH 1.2, 4.0, and 6.0, 0.5 g of charcoal resulted in a moderate increase in the amount of drug adsorbed. As seen in Figs. 1–3, the amount of drug adsorbed by 0.5–1.0 g of charcoal is approximately the same.

As observed from Fig. 4, the amount of phenobarbital adsorbed by 0.1 g of charcoal from a solution of pH 8.0 is significantly lower than from solutions of pH 1.2, 4.0, and 6.0. Even though 0.3 g of charcoal recorded a significantly higher increase in the amount of drug adsorbed from a solution of pH 8.0, the increase was not as high as from solutions of pH 1.2, 4.0, and 6.0. For other amounts of charcoal as 0.5, 0.7, 0.9, and 1.0 g in solutions of pH 8.0, the increase was gradual and about equally distributed as far as the increment in the amount of charcoal is concerned. At pH 8.0, most of the drug exists as ionized molecules. Since adsorption onto charcoal is greater for nonionized compounds, low adsorption values obtained from a solution of pH 8.0 may be due to this reason.

It is evident that most of the phenobarbital was adsorbed onto charcoal within 60 min and adsorption was practically complete with all amounts of charcoal (except 0.1 and 0.3 g) over the pH range tested. Hpwever, the percent of phenobarbital adsorbed was dependent upon the quantity of charcoal used. The adsorption by 0.1 and 0.3 g of charcoal was not complete even at the end of 120 min, but at the 0.5-g level the adsorption was almost complete at the end of 60 min. Further increments of 0.2 g above 0.5 g did not appreciably increase the extent of adsorption in adsorbing the phenobarbital from 50 ml of standard phenobarbital sodium solution.

Desorption studies were performed in solutions of various pHs containing different amounts of charcoal to determine how much of the phenobarbital once adsorbed would be desorbed as a result of a change in the pH of the eluting medium. Table I illustrates the effect of variation of the eluting media pH on the extent of desorption at the end of 2 hr from various concentrations of charcoal. It is evident that 0.1 g of charcoal was not sufficient to hold all of the drug, and ~50% of the drug was eluted. But with 0.5 g and higher amounts of charcoal, the extent of desorption was <5%. These findings substantiate previous results (10, 11) which suggested that the charcoal–poison complex remains stable throughout the GI tract. In general, desorption from lower amounts of charcoal was essentially complete within 15 min, decreased with increasing amounts of charcoal, and was independent of pH. Therefore, it can be summarized that 0.5–0.7 g of charcoal seems to be the optimum quantity at which minimum desorption takes place. Further increments in the amount of charcoal did not significantly contribute to holding the drug particles.

Langmuir adsorption isotherms for the adsorption of phenobarbital calculated as phenobarbital sodium onto charcoal were determined from solutions of various pH. The Langmuir isotherm may simply be defined as:

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

where c is the concentration of phenobarbital sodium in milligrams per milliliter at equilibrium, x is the amount of drug adsorbed in milligrams per m milligrams of charcoal. The isotherm constants n and b were determined by linear regression analysis at each pH value. Parameters presented in Table II substantiate the theory that no change in binding is indicated at different pH levels (with the numbers under the adsorption capacity column being practically the same), and that these results can possibly be construed to indicate the stability of the charcoal–drug complex throughout the physiological pH range.

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Determination of Ciramadol in Plasma by Gas–Liquid Chromatography

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Received December 14, 1981, from the *Drug Metabolism Subdivision, Wyeth Laboratories, Inc., Philadelphia, PA 19101.* Accepted for publication February 25, 1982.

Abstract □ An analytical method for determining ciramadol concentrations in plasma was developed and evaluated for its specificity, precision, linearity, and sensitivity. GLC–electron capture detection of a dipentafluorobenzoyl derivative of the drug was used for quantitation. An isomer of the drug served as an internal standard. Resulting mean ratios of the peak height of derivatized drug to that of derivatized internal standard varied with a coefficient of variation that ranged from 3.8 to 11.1%. The mean ratio was linearly related to ciramadol content (8.75–175 ng) with a correlation coefficient >0.999. The minimum quantifiable concentration was 4 ng/ml with a 2-ml specimen. An application of this method is presented.

Keyphrases □ Ciramadol—analgesics, GLC determination in plasma □ GLC—determination of ciramadol in plasma, analgesics □ Analgesics—GLC determination of ciramadol in plasma

Ciramadol [(−)*cis*-2-(α-dimethyl (I) amino-*m*-hydroxybenzyl)cyclohexanol hydrochloride] is a potent analgesic when administered to animals, and indications of its effectiveness in relieving mild to moderate pain in malignant disease and moderate to severe postoperative pain in humans have been reported (1–3). During previous metabolic disposition studies (4), it was recognized that a very sensitive assay for the drug in biological fluids would be required during preclinical studies and especially when clinical bioavailability studies were performed. Maximum concentrations in the plasma of rats given single 1-mg/kg intragastric doses of the drug were highest (105 ng/ml) at the earliest sampling time (15 min), and when studies were conducted in rhesus monkeys, maximum concentrations in plasma never exceeded 4 ng/ml after a similar dose. In the current report, an assay for ciramadol, which meets the desired requirements, is presented. It is based on the electron-capturing capability of a dipentafluorobenzoylated derivative following a GLC separation.

EXPERIMENTAL

Standards and Reagents—Ciramadol (I) and the internal standard [*trans*-2-(α-dimethylamino-*p*-hydroxybenzyl)cyclohexanol hydrochloride hemiethanolate, II] were synthesized in these laboratories[1]. Deionized water was prepared by passing distilled water through an ion-exchange system[2]. Pentafluorobenzoyl chloride and all other reagents and solvents were purchased from commercial sources.

[1] Dr. J. P. Yardley, Medicine Chemistry Section II, Wyeth Labs., Inc., Radnor, Pa.
[2] Bion Exchanger System, Pierce Chemical Co., Rockford, Ill.

Pentafluorobenzoyl chloride was purified by distillation prior to use. The fraction boiling at 158–159° was collected and stored in a sealed container at 5°.

Calibration standards were prepared from a stock solution containing 1 mg of drug/ml of deionized water. Subsequently, solutions containing 0.01, 0.02, 0.05, 0.1, and 0.2 μg of drug/100 μl, or, respectively, 8.75, 17.5, 43.75, 87.5, and 175 ng of free base/100 μl were prepared. All concentrations of ciramadol in biological fluids are expressed as free base.

The concentration of the internal standard solution prepared in water was 100 ng/100 μl.

Preparation of Pentafluorobenzoyl Derivatives—One gram of ciramadol (as free base) was dissolved in 80 ml of benzene and 30 ml of pyridine. To the solution, 2.5 g of pentafluorobenzoyl chloride was added dropwise with stirring at 25° for 2 hr. The reaction mixture was washed with 100 ml each of water, 10% Na_2CO_3, water, 0.5 *N* H_2SO_4, 10% Na_2CO_3, and water. The organic layer was separated, dried over anhydrous sodium sulfate, treated with 2 g of charcoal[3], filtered, and evaporated. A pale yellow syrup was obtained. After treating again with charcoal the syrup was analyzed.

Anal.—Calc. for $C_{29}H_{21}NO_4F_{10}$: C, 54.64; H, 3.32; N, 2.20. Found: C, 54.64; H, 3.44; N, 2.27.

Compound II (200 mg) was dissolved in 16 ml of benzene and 6 ml of pyridine. To the solution, 0.5 g of pentafluorobenzoyl chloride was added dropwise with stirring at 25° for 2 hr. The reaction mixture then was washed sequentially (20-ml portions) and treated with charcoal as described above. After the solvent was removed under reduced pressure, a syrup was obtained. A pale yellow crystalline product precipitated after the syrup was dissolved in warm ethanol and cooled at 4°. The precipitate was collected, recrystallized from ethanol, and dried in air. Yield: 75 mg.

Anal.—Calc. for $C_{29}H_{21}NO_4F_{10}$: C, 54.64; H, 3.32; N, 2.20. Found: C, 54.43; H, 3.49; N, 2.65.

Mass Spectrometry—Mass spectrometric analysis of the two derivatives was performed on a mass spectrometer[4] (electron beam, 70 eV; source temperature, 200°) equipped with a data system[5]. Samples were examined by direct introduction in the electron-impact mode.

Analysis of Ciramadol in Plasma—*Extraction and Derivatization*—Standards (0.01, 0.02, 0.05, 0.1, and 0.2 μg of ciramadol) were added to a series of 16 × 125-mm culture tubes (polytef lined[6] screw cap) containing 1 ml of control (drug free) dog plasma. To each sample was added

[3] Norit, Fisher Scientific Co., Fair Lawn, NJ 07416.
[4] AEI-MS 902, Associated Electronic Ind., Ltd., U.K.
[5] Nova 3 Computer system with DS-50S software, Data General Corp., Southboro, Mass.
[6] Teflon, DuPont Co., Wilmington, Del.

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