In Vitro Interaction of Quinidine with Kaolin and Pectin

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Abstract □ The adsorption of quinidine onto kaolin was studied as a function of pH in aqueous solutions in which the ionic strength was adjusted to 0.1. The interaction of quinidine with pectin also was investigated in water and in phosphate buffer; the buffer pH and ionic strength were adjusted to 6.5 and 0.1, respectively. The *in vitro* results indicated that quinidine was adsorbed onto kaolin. At the highest concentration studied, the extent of adsorption increased from 3.64 mg of quinidine adsorbed/g of adsorbent at pH 2.4 to an average of 5.81 mg/g in the pH 5.5–7.5 range. In the presence of electrolytes, the interaction of quinidine with pectin was relatively small (3–13% bound) as compared to studies performed in water (66–90% bound). The data indicate that some quinidine may be adsorbed when this drug is administered concurrently with kaolin–pectin preparations.

Keyphrases □ Quinidine—interactions with kaolin and pectin, *in vitro* □ Kaolin—interactions with quinidine, *in vitro* □ Pectin—interactions with quinidine, *in vitro* □ Antiarrhythmic agents—quinidine, interactions with kaolin and pectin, *in vitro*

Quinidine is used in the treatment of cardiac arrhythmias, and its therapeutic effect is associated with maintaining serum levels of 2.0–6.0 μg/ml (1, 2). Side effects frequently encountered with these therapeutic levels include GI disturbances such as diarrhea (2–4). Pharmaceutical preparations containing both kaolin and pectin are available without prescription and frequently are used to treat diarrhea. Previous *in vitro* studies demonstrated that kaolin adsorbs various alkaloids (5), phenothiazines (6, 7), and cimetidine (8). *In vivo* studies showed that kaolin–pectin preparations concomitantly administered with lincomycin (9) or digoxin (10, 11) decreased the rate and the extent of absorption of these drugs.

Since kaolin adsorbs quinine *in vitro* (5), the cinchona alkaloid quinidine may be adsorbed by kaolin in the kaolin–pectin antidiarrheal preparations. Quinidine was reacted with polygalacturonic acid to form quinidine polygalacturonate (12). Since polygalacturonic acid is derived from pectin, complexation of quinidine with pectin also may occur, thus affecting the amount of quinidine available for absorption in the GI tract.

The purpose of this study was to determine the extent of quinidine adsorption onto kaolin as a function of physiological pH as well as the degree of quinidine–pectin interaction.

EXPERIMENTAL

Reagents—Quinidine sulfate[1], 37% hydrochloric acid[2], monobasic sodium phosphate[3], dibasic sodium phosphate[3], anhydrous sodium sulfate[4], kaolin NF[4], and pectin[1] were used as received. The cellulose membrane tubing[5] employed had a molecular weight cutoff of 12,000–14,000.

Analytical Method—Quinidine sulfate concentrations in test solutions were determined using a fluorescence spectrophotometer[6] with a xenon power supply[7] and a recorder[8]. An excitation wavelength of 350 nm and an emission wavelength of 443 nm were used. By using a sensitivity setting of 4 and a concentration range of 0.2–1.0 μg/ml, a standard curve (r = 0.999) was obtained as expressed by:

$$\%F = 100.0C + 0.175 \qquad \text{(Eq. 1)}$$

where $\%F$ is the emission intensity in percent and C is the quinidine sulfate concentration in micrograms per milliliter. To minimize daily variation, a standard curve was obtained on a regular basis using controls subjected to the same conditions as the experimental samples.

Determination of Equilibrium Time for Quinidine Adsorption onto Kaolin—One-gram quantities of kaolin were weighed accurately into clean, dry, amber-glass bottles. Then 40.0 ml of quinidine sulfate solution was added. The quinidine concentrations in the time study were 5.0 and 40.0 mg/100 ml, representing the lowest and highest concentrations utilized. The bottles were capped tightly, placed in a thermostatically controlled water bath, and agitated at 37.0 ± 0.5°.

Samples were withdrawn at 6.0, 12.0, and 24.0 hr and were filtered immediately through a 0.22-μm membrane disposable filter[9]. The filtered solution was diluted and analyzed for the remaining quinidine sulfate content. Controls, containing identical concentrations of quinidine sulfate without kaolin, were treated in the same manner to check for any loss of quinidine due to degradation or adsorption onto the glassware and filter. A blank solution that contained kaolin without quinidine sulfate was prepared similarly.

Kaolin Adsorption Studies—The adsorption of quinidine onto kaolin was studied as a function of pH. Solution I consisted of 0.01 *M* HCl with a final pH of 2.4. Solutions II, III, and IV were phosphate buffers with final pH values of 5.5, 6.5, and 7.5, respectively. All solutions were adjusted to a final ionic strength of 0.10 by the addition of anhydrous sodium sulfate.

In all solutions studied, 40.0 ml of quinidine sulfate solution of 5.0, 10.0, 15.0, 20.0, 30.0, and 40 mg/100 ml was added to 1.0 g of kaolin in dry, amber-colored bottles. All samples were subjected to the same conditions and treated like those in the time study. All samples were left to equilibrate for 24.0 hr.

Pectin Studies—The interaction of quinidine with pectin was studied in water and in Solution III using membrane dialysis techniques similar to those reported previously (13, 14). Membrane bags were prepared from

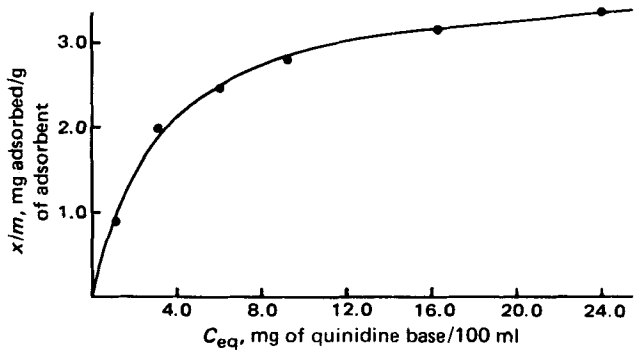

Figure 1—*Typical isotherm for the adsorption of quinidine onto kaolin in Solution I (pH 2.4) at 37°.*

[1] Sigma Laboratories, St. Louis, Mo.
[2] Mallinckrodt, St. Louis, Mo.
[3] Fisher Scientific Co., Fair Lawn, N.J.
[4] Matheson, Coleman and Bell, Norwood, Ohio.
[5] Spectropore-2, Spectrum Medical Laboratories, Los Angeles, Calif.
[6] Perkin-Elmer model 204.
[7] Perkin-Elmer model 150.
[8] Perkin-Elmer model 165.
[9] Millex, Millipore Corp., Bedford, Mass.

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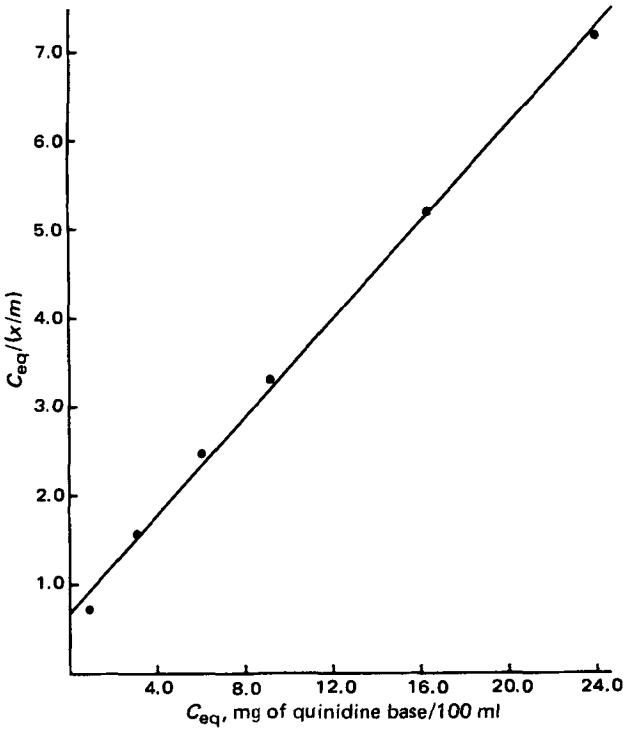

Figure 2—*Linear Langmuir plot for the adsorption of quinidine onto kaolin in Solution I (pH 2.4) at 37°, r = 0.999.*

cellulose tubing and were filled with 40.0 ml of pectin solution. Concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0% pectin solution in water or in Solution III were used. The bags were clamped securely at the bottom and tied twice at the top to prevent leakage.

After inspection for leakage, the bags were immersed in a jar containing 60 ml of a 40.0-mg/100 ml quinidine sulfate solution either in water or in a buffered Solution III. The jars were capped tightly and placed in a shaking water bath thermostatically controlled to 37.0 ± 0.5°. The jars were allowed to agitate for 6.0 hr, a time previously determined as sufficient for achieving equilibrium. Additional membrane bags containing the appropriate solution were included in each study to check any loss of quinidine due to binding to the membrane and clamp.

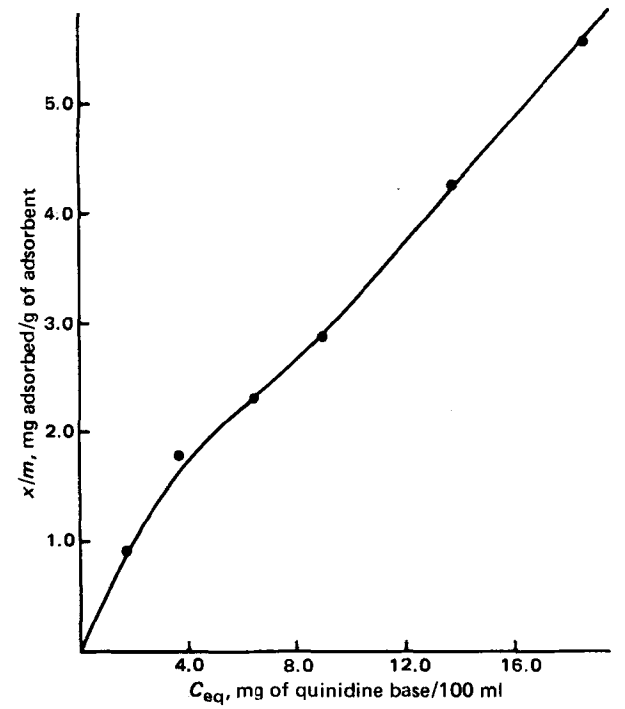

Figure 3—*Isotherm illustrating the adsorption of quinidine onto kaolin in Solution II (pH 5.5) at 37°.*

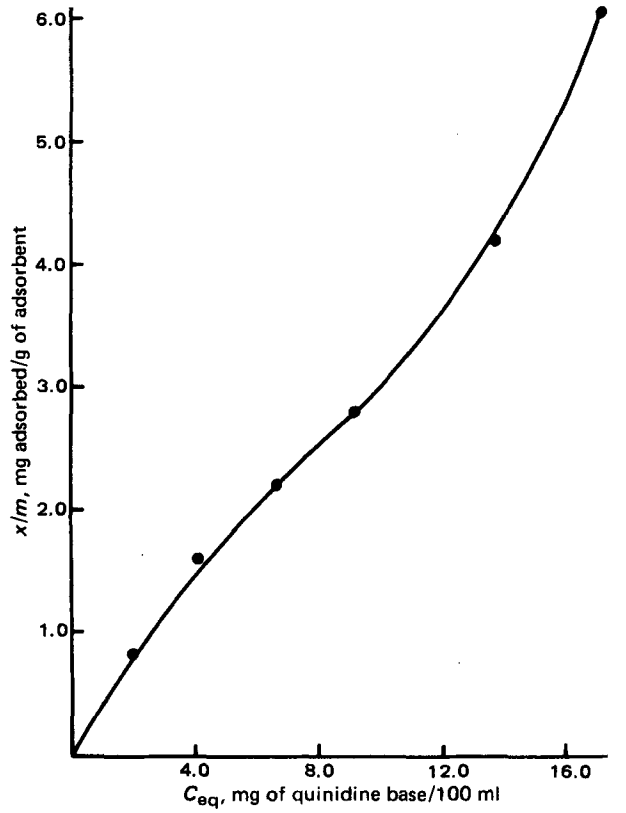

Figure 4—*Isotherm illustrating the adsorption of quinidine onto kaolin in Solution III (pH 6.5) at 37°.*

Four runs were performed for each pectin concentration. After achieving equilibrium and appropriate dilution, the quinidine sulfate contents both inside and outside the bag were determined spectrofluorometrically.

RESULTS AND DISCUSSION

The kaolin time study indicated that equilibrium was achieved between 6.0 and 12.0 hr. To ensure equilibrium, all samples were allowed to agitate in the water bath for 24.0 hr. The membrane filter had no effect on the quinidine sulfate concentration based on assays performed prior to and after filtration. Furthermore, the lack of any significant decrease in the quinidine sulfate concentration in the controls indicated the apparent absence of adsorption of the drug onto the glassware and degradation during equilibration.

The results of the kaolin adsorption studies in Solution I were evaluated according to the Langmuir equation (Eq. 2):

$$\frac{x}{m} = \frac{k_1 k_2 C_{eq}}{1 + k_1 C_{eq}} \qquad \text{(Eq. 2)}$$

where x/m is the weight, in milligrams, of quinidine base adsorbed per gram of kaolin; C_{eq} is the concentration of quinidine base, in milligram percent, remaining in solution at equilibrium; k_1 is the adsorption coefficient; and k_2 is the maximum amount of drug adsorbed to form a monolayer under the experimental conditions. Figure 1 is a typical Langmuir isotherm obtained by plotting x/m *versus* C_{eq} and illustrates the formation of a quinidine monolayer on kaolin. Figure 2 is the fitted plot according to the following linear form of Langmuir:

$$C_{eq}/(x/m) = C_{eq}/k_2 + 1/k_1 k_2 \qquad \text{(Eq. 3)}$$

The maximum adsorptive capacity calculated from the slope of the line is 3.64 mg/g.

Ionic strength was adjusted with anhydrous sodium sulfate since preliminary studies showed that a high concentration of chloride ions, either in the form of hydrochloric acid (0.1 *M*) or sodium chloride for adjusting the ionic strenth to 0.1, caused quenching and interfered with the assay. The low concentration of hydrochloric acid (0.01 *M*) did not present any analytical problem. With quinidine sulfate concentrations up to 40.0 mg/100 ml, the results of adsorption studies in Solution II indicate that adsorption is not limited to the formation of a monolayer as

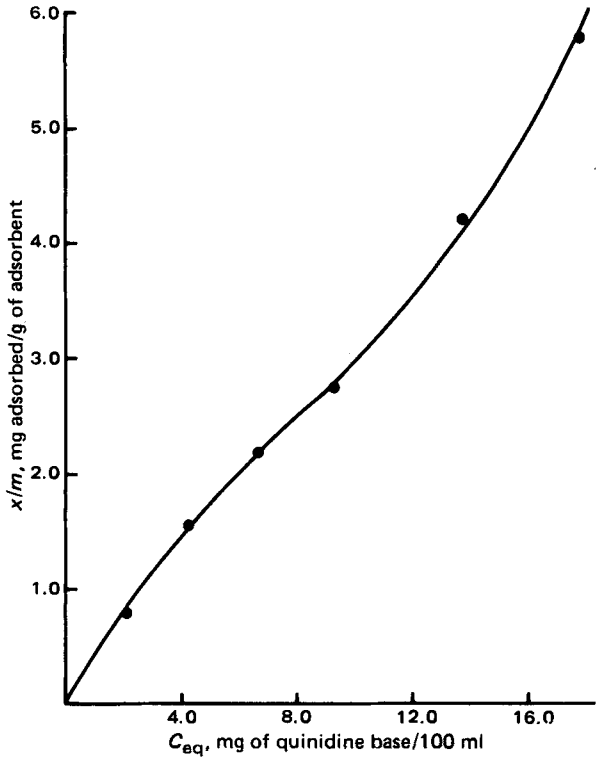

Figure 5—*Isotherm illustrating the adsorption of quinidine onto kaolin in Solution IV (pH 7.5) at 37°.*

observed in Solution I. This result is shown in Fig. 3, which indicates that multilayered adsorption occurred. Similar results were obtained from studies in Solutions III and IV (Figs. 4 and 5, respectively). Langmuir linearity was established for Solution II in quinidine sulfate concentrations between 1.0 and 3.0 mg/100 ml (Fig. 6). In this same low concentration range, linearity was not observed for Solutions III and IV.

These results indicate that adsorption of quinidine onto kaolin varies with pH. The apparent multilayered process that occurred in Solutions II–IV indicates that drug adsorption continues at higher pH values. Further experimentation is underway to explain this behavior.

Since increasing electrolyte concentrations may increase the adsorptive capacity of kaolin (7), it is not surprising that there are no apparent differences in the isotherms (Figs. 3–5) since the ionic strength was kept constant in all solutions. For example, at an initial concentration of 32.4 mg of quinidine base/100 ml (equivalent to 40 mg of quinidine sulfate/100 ml in solution) the calculated x/m values are 5.57, 6.07, and 5.79 mg/g for Solutions II, III, and IV, respectively. Therefore, at quinidine sulfate concentrations up to 40.0 mg/100 ml, the amount of drug adsorbed per

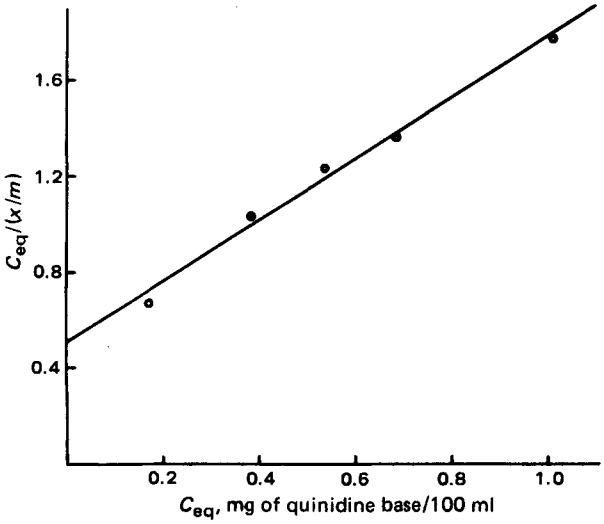

Figure 6—*Linear Langmuir plot for the adsorption of quinidine onto kaolin at low concentrations in Solution II (pH 5.5) at 37°, r = 0.994.*

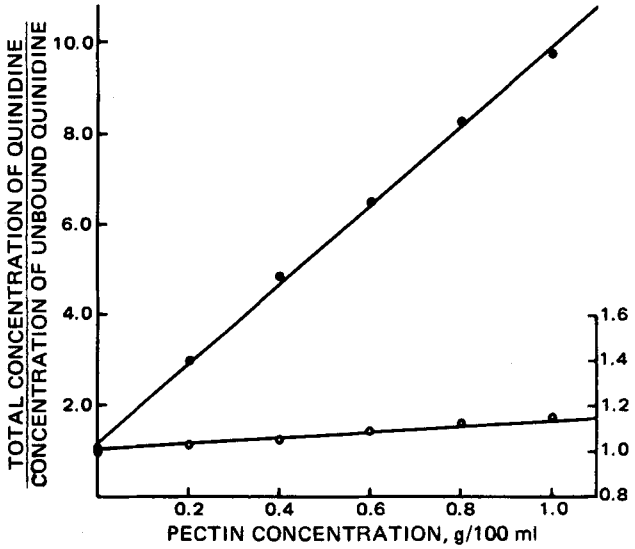

Figure 7—*Interaction between quinidine and pectin at 37°. Key: ●, water (r = 0.999); and ○, pH 6.4 buffer at an ionic strength of 0.1 (r = 0.998).*

gram of adsorbent increases as the pH is raised from acidic pH, simulating the stomach environment, to pH 5.5–7.5, which simulates the intestinal environment. The results of the present study parallel the findings of Remon *et al.* (15) who reported that the adsorption of quinidine onto kaolin increases with pH. Their results were obtained under different *in vitro* conditions with much higher quinidine sulfate concentrations. Thus, any firm comparisons between the two studies are difficult.

The data of the pectin study were analyzed by previously reported methods (14). The calculated coefficient of variation for identical runs was 1–4%. Figure 7 shows marked complexation between quinidine and pectin in unbuffered aqueous medium. The extent of binding of quinidine ranged between 66 and 90% at a pectin concentration between 0.2 and 1%. Binding was reduced markedly when the study was repeated in Solution III. The extent of binding ranged between 3 and 13% in the same pectin concentration range (Fig. 7). According to these data, complexation studies performed in water without electrolytes could be misleading. Studies utilizing electrolyte solutions, such as Solution III, may be more representative of an *in vivo* situation. The same pattern of interaction was observed by Remon *et al.* (15) who studied quinidine sulfate complexation with 1% pectin in water and in 0.01 *M* HCl. They reported that quinidine sulfate was 40% bound to pectin in water and was 5% bound in acidic solution. In the current study, ~90% of quinidine was bound by 1% pectin in water. This difference may be accounted for by the higher quinidine concentrations (500 mg/100 ml) used previously (15).

The results of this *in vitro* study indicate that kaolin is capable of adsorbing quinidine and that adsorption increases with pH. Pectin forms a complex with quinidine; however, the effect is less pronounced in Solution III than in water. Although complexation with pectin may afford a controlled and more uniform absorption of quinidine as shown for quinidine polygalacturonate (12), the data suggest that this effect may be minimal. Kaolin and pectin contained in antidiarrheal preparations may alter absorption of the drug from the GI tract. Relative bioavailability studies should be carried out to determine the significance of this interaction.

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Quantification of Phencyclidine in Mainstream Smoke and Identification of Phenylcyclohex-1-ene as Pyrolysis Product

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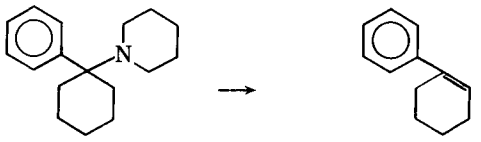
Abstract □ Parsley cigarettes containing [^{3}H]phencyclidine were machine smoked, and the mainstream smoke was trapped in glass wool filters. Radioactivity was extracted from these filters with chloroform. The average recoveries of radioactivity were 76, 85, 70, and 69% for cigarettes containing 3, 10, 30, and 50 mg of [^{3}H]phencyclidine hydrochloride, respectively. TLC and GLC–mass spectrometry were employed to identify and quantify compounds in the filter extracts. Approximately one-half of the recovered radioactivity represented a pyrolysis product, phenylcyclohex-1-ene. Formation of this product involved loss of piperidine from phencyclidine. Piperidine, which was not radiolabeled, also may appear in smoke intact. The remainder of the radiolabeled material represented unchanged phencyclidine. Therefore, the percentage of [^{3}H]phencyclidine delivered was ~40% of the amount smoked. This result was independent of puff frequency and quantity of phencyclidine hydrochloride smoked over the range tested. The [^{3}H]phencyclidine delivery was compared to the quantities of [^{3}H]-Δ^9-tetrahydrocannabinol and [^{3}H]nicotine delivered in mainstream smoke. The recovery of unchanged [^{3}H]-Δ^9-tetrahydrocannabinol from placebo marijuana cigarettes injected with a solution containing 3 mg of Δ^9-tetrahydrocannabinol was 60%. Tobacco cigarettes injected with [^{3}H]nicotine yielded 70% unchanged nicotine in mainstream smoke.

Keyphrases □ Pyrolysis—formation of phenylcyclohex-1-ene from phencyclidine in smoke □ Phencyclidine—identification of pyrolysis product phenylcyclohex-1-ene in smoke □ Phenylcyclohex-1-ene—pyrolysis product of phencyclidine identified in smoke □ Smoke—identification of phenylcyclohex-1-ene, a pyrolysis product of phencyclidine

Phencyclidine [1-(1-phenylcyclohexyl)piperidine] has gained popularity as a drug of abuse, probably because it produces euphoria, dissociation, and hallucinations (1). Smoking phencyclidine-impregnated spices is currently a major method of abuse. The high temperature attained in a burning cigarette raises the possibility that phencyclidine may evolve pyrolysis products when smoked. Phencyclidine is converted to phenylcyclohex-1-ene in the gas chromatograph at low temperatures (2) (Scheme I), which may have led to the conclusion that phenylcyclohexene is a metabolite of phencyclidine (3). Preliminary results indicate that phenylcyclohexene also is formed during smoking (4). In this study, the amount of phencyclidine delivered in mainstream smoke was determined and the pyrolysis products were identified and quantified.



phencyclidine → phenylcyclohex-1-ene

Scheme I

EXPERIMENTAL

Drugs—Phencyclidine hydrochloride[1], piperidinocyclohexane carbonitrile[1], [*phenyl*-1-^{3}H(*n*)]phencyclidine[1] (17 Ci/mmole), Δ^9-tetrahydrocannabinol[1], and [1′,2′-^{3}H]-Δ^9-tetrahydrocannabinol[1] (43 mCi/mmole) were used. [*pyrrolidinyl*-4′,4-^{3}H]Nicotine (4.7 Ci/mmole) was synthesized according to the procedure of Vincek *et al.* (5).

Preparation of Cigarettes—Cigarettes containing phencyclidine were prepared using commercially available parsley flakes[2], 55-mm cigarette tubes[3], and a cigarette machine[4]. Each cigarette (550–600 mg) was prepared immediately prior to its use by injection with 0.5 μCi of [^{3}H]-phencyclidine and 3, 10, 30, or 50 mg of phencyclidine hydrochloride in volumes of 50, 50, 150, or 200 μl of ethanol, respectively. Filterless cigarettes[5] (84 mm) were injected with 0.5 μCi of [^{3}H]nicotine in 50 μl of ethanol, and placebo marijuana cigarettes[1] were injected with 0.5 μCi of [^{3}H]-Δ^9-tetrahydrocannabinol and 3 mg of Δ^9-tetrahydrocannabinol in 50 μl of ethanol. All drug solutions were injected axially and evenly throughout the middle 35 mm of the cigarettes and allowed to dry before smoking.

Procedure—Cigarettes were inserted in a holder and attached to the smoking apparatus (Fig. 1). Smoking was effected by negative pressure provided by water aspiration, and smoke was collected on two contiguous filters (each consisting of 1–1.25 g of glass wool stuffed in 16 cm of 0.635-cm i.d. tubing[6]). Interfaced between the filters and vacuum source were an empty trap (for back-siphoning) and a trap containing 10 ml of chloroform.

Puffing was accomplished with an electric switching valve[7] that opened and closed the system to the vacuum every 5.8 sec. Puff frequency was altered by replacement of a capacitor in the circuit board controlling the valve. A vacuum regulator[8] was set to permit delivery of 45 ml of smoke/5.8-sec puff duration. After an entire cigarette was smoked, each

[1] National Institute on Drug Abuse.
[2] McCormick and Co., Baltimore, Md.
[3] Dominion Cigarette Tube Co., Montreal, Canada.
[4] Premier Supermatic, Central Tobacco Manufacturing Co. Ltd., Montreal, Canada.
[5] Pall Mall, American Tobacco Co., Richmond, Va.
[6] Tygon Tubing and Molded Products, Akron, Ohio.
[7] Skinner, New Britain, Conn.
[8] Fairchild, Winston-Salem, N.C.

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