Percutaneous Absorption and Disposition of Iodochlorhydroxyquin in Dogs

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Abstract □ The percutaneous absorption and disposition of iodochlorhydroxyquin (5-chloro-7-iodo-8-quinolinol; I) from a 3% cream were studied in five dogs over a 28-d topical treatment period. Plasma levels, determined by HPLC, were 0.275–0.525 μg/mL. The steady-state elimination rate of total I in urine was 2.4–3.0 mg/d. The apparent elimination rate constant and half-life were 0.25 ± 0.05 d^{-1} and 3.1 ± 0.5 d, respectively. Greater than 50% of topically applied I was absorbed over 16 h. Occlusion of the skin without the drug indicated that the skin acted as a reservoir for the drug. Feces analysis for iodochlorhydroxyquin from one dog showed that 27.1 ± 8.5 mg/d was eliminated *via* this route. Tissue levels of I 15 d after the 28-d topical treatment were 0.7 μg/g of liver, 0.2 μg/g of kidney, and 0.8 μg/g of mesenteric fat. The apparent rate constants of plasma level decline after a 100-mg iv bolus dose of I were $\alpha = 3.9$ h^{-1} and $\beta = 0.6$ h^{-1}. The urinary elimination after intravenous administration was biphasic. The rate constant for the slow elimination phase was 0.4 ± 0.1 d^{-1}, and the half-life was 2.0 ± 0.5 d. The primary neurological symptoms observed during topical treatment were ataxia and hind limb paralysis. Microscopic examination revealed liver necrosis. A weight loss of 15.3 ± 2.7% was also observed over the 28-d topical treatment period. The results indicate that significant percutaneous absorption of I occurs, and that chronic high-dose topical treatment may lead to toxicity.

Keyphrases □ Iodochlorhydroxyquin—percutaneous absorption and disposition in dogs □ Absorption—percutaneous, disposition of iodochlorhydroxyquin in dogs □ Neurotoxicity—percutaneous absorption and disposition of iodochlorhydroxyquin in dogs

Iodochlorhydroxyquin (5-chloro-7-iodo-8-quinolinol; I), also known as clioquinol, is a dihalogenated derivative of 8-hydroxyquinoline. It is used widely as an amebicidal agent, and as an antibacterial and antifungal agent for the treatment of various dermatological disorders. Due to its neurotoxicity, the drug is no longer given orally in the United States and Japan, but it is still used orally in many countries (1, 2). Iodochlorhydroxyquin is still used topically for diaper rash and other skin disorders and is presumed to undergo little or no percutaneous absorption. Tateishi and Otsuki (3) first reported that oral administration to dogs, cats, and monkeys resulted in the occurrence of a myelo-opticneuropathy. The neurological symptoms seen in these animals were comparable with those seen in patients suffering from subacute myelo-opticneuropathy (3).

Worden and Heywood (4) have reported hind limb and proprioceptive reflexes and yellow staining of the fur when dogs were treated orally with 250–400 mg of I per kg of body weight daily for 25 weeks. The minimum serum levels of I at the beginning of intoxication during chronic studies in adult dogs were 6–22.6 μg/mL when given 100 mg/kg/d orally (5).

Hansson (6) has studied the oral absorption of the drug in normal humans and reported that 3–5% of a given oral dose was recovered in the urine. Berggren and Hansson (7) have observed that the proportion of an oral dose of halogenated hydroxyquinoline which is absorbed was as high as 46%.

Iodochlorhydroxyquin is believed to undergo limited absorption through the skin, although few data are available on the extent of this absorption. A child with generalized psoriasis treated with ointment containing I was reported to have 18.1

mg/100 mL of the conjugated drug in the urine (8). Fischer and Hartvig (9) treated four patients with widespread dermatitis of an unstated type with an ointment containing 3% I, and serum levels of I were estimated by electron-capture GC to be 0.8–1.2 μg/mL. In this study, we examined the percutaneous absorption and excretion of iodochlorhydroxyquin in dogs.

EXPERIMENTAL SECTION

Animals and Procedure—Five male mongrel dogs (weight, 16.5–19 kg) were used. The dogs were fed a solid diet[1] and had free access to water. All dogs were treated for parasites and vaccinated against hepatitis. The animals were maintained for at least 1 week before initiating the studies. Five grams of 3% iodochlorhydroxyquin cream[2] was applied topically twice daily at 8 a.m. and 4 p.m. for 28 d over a 200-cm^2 area on the back of each dog after the area was shaved. The area was occluded with plastic wrap[3] and gauze. Before application of each new dose of I, the skin was cleansed with water and dried. Blood samples were drawn at the following times during treatment: 0, 1, 2, 4, 6, 8, 10, 12, and 24 h and 2, 3, 4, 7, 14, 21, and 28 d. Blood samples were obtained during the first week through an indwelling catheter placed in the jugular vein. The tubing was tunneled under the skin from the jugular vein to the back behind the head and exteriorized. The exposed end of the catheter was covered with a padded collar around the neck to prevent damage to the catheter by the dog. Each dog was also inserted into an elastic stockinette which enclosed the collar, neck, and thorax and prevented scratching of the jacketed area. The patency of the catheter was maintained by flushing with normal saline and filling with heparin sodium (1000 USP U/mL) every day. A blood sample was withdrawn and discarded, and a 3-mL sample of blood was then withdrawn for analysis.

At the end of the first week of treatment, the catheter was closed. For the subsequent time intervals, blood samples were drawn from a foreleg using heparin as the anticoagulant. Plasma was separated by centrifugation at 3000×*g* for 10 min and refrigerated or frozen if the samples were not analyzed immediately. No plasma sample was kept for more than 3 days before analysis. Urine samples taken every 24 h were collected at the following times during treatment by using metabolism cages: 1, 2, 3, 4, 5, 6, 7, 10, 14, 21, and 28 d. Feces were collected every 24 h from one dog during the 28-d treatment on days 0, 1, 7, 14, 21, and 28.
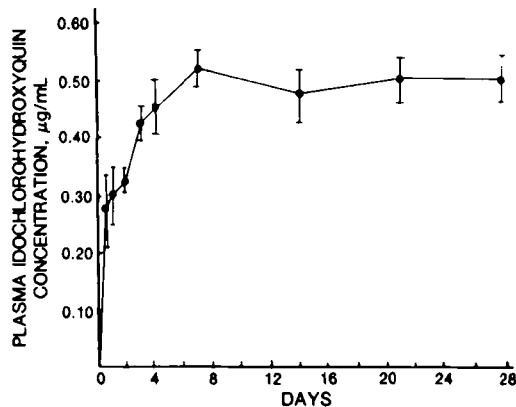

Figure 1—*Mean plasma level ± SD of nonconjugated iodochlorhydroxyquin in five dogs after topical treatment.*
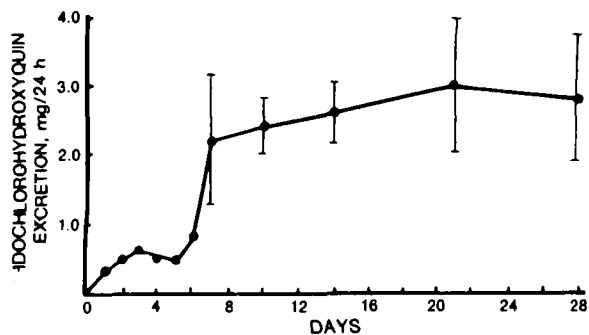
Figure 2—*Mean rate of excretion ± SD of total iodochlorhydroxyquin with time over 28 d of topical treatment of five dogs.*

After the 28-d topical treatment, blood and urine samples were collected at 2, 3, 4, 6, 8, 10, and 12 d posttreatment. Following this 12-d posttreatment interval, the backs of the dogs were again occluded with plastic wrap and gauze without drug, and blood and urine samples were obtained for an additional 6 d. The general condition of the dogs was examined daily, and the animals were weighed weekly.

Two months after topical treatment with I, three dogs were given a single bolus dose of 100 mg of I intravenously dissolved in 2.0 mL of dimethyl sulfoxide and 0.50 mL of ethanol. Blood samples were drawn at 15, 30, 45, 60, 120, and 180 min, and urine samples were collected each 24 h for 6 d.

Microscopic Examinations—The optic nerve from one dog that was killed after 28 d of topical treatment with I was isolated and fixed with glutaraldehyde and examined by electron microscopy. Kidney and liver tissues obtained from the dogs following sacrifice (after the intravenous treatment with I) were examined microscopically. Formalin (10%) was used to fix the tissues. Liver and kidney sections were also fixed and examined from one dog which died 15 d posttreatment.

Sample Analysis—The plasma concentration of nonconjugated iodochlorhydroxyquin was determined by HPLC after ether extraction by using a C_{18} reverse-phase column (10). The total urinary excretion of I after hydrolysis of conjugated metabolites according to Chen *et al.* (11) was assayed by a previously described HPLC procedure (12). The nonabsorbed I remaining on the backs of the dogs after topical treatment was also analyzed by HPLC as previously reported (13).

RESULTS AND DISCUSSION

Iodochlorhydroxyquin (I) cream was applied twice daily for 28 d, and the mean plasma level of nonconjugated I from five dogs were determined and plotted against time (Fig. 1). A steady-state plasma level was achieved in 4–5 d at ∼0.5 μg/mL. Our results demonstrated that the drug is readily absorbed through the skin and presumably passes directly into the systemic circulation where easily measurable levels of the nonconjugated form occur after topical treatment (Fig. 1). Plasma levels could be detected 12 h after initial application, and accumulation of the drug in plasma did not occur.

During topical treatment, 24-h urine samples were collected, and the mean rate of elimination was plotted against time (Fig. 2). After day 7, the elimination rate reached steady state and ranged from 2.4 to 3.0 mg/24 h. In previous studies, it has been shown that >90% of the drug excreted in the urine
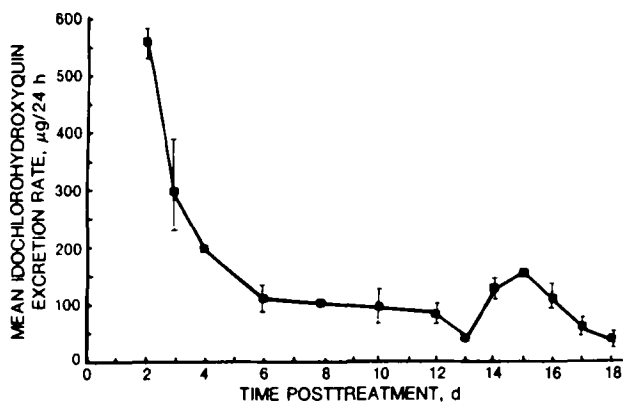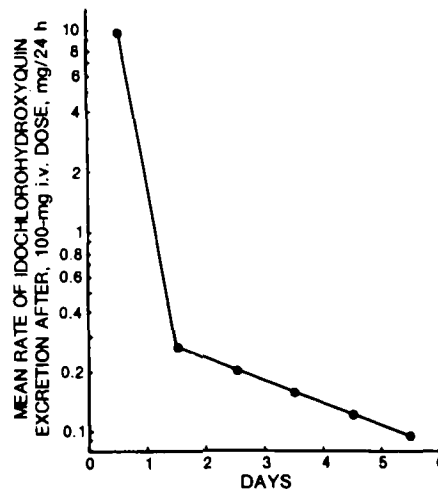

Figure 4—*Logarithmic plot of the mean excretion rate of iodochlorhydroxyquin with time after discontinuation of topical treatment in four dogs.*

is in the conjugated form, either as the glucuronide or sulfate (5). Therefore, the total amount of the drug eliminated in the urine during topical application was determined after hydrolysis of the conjugates. In spite of continuing topical treatment, the plasma levels and urine excretion rates remained constant, suggesting that the drug was being eliminated *via* another route and/or being stored in various tissues of the dogs. Therefore, feces from one dog were collected and analyzed over 28 d of topical treatment. The average total excreted in the feces was 27.1 ± 8.5 mg/24 h, indicating that the drug was being excreted largely by the fecal route as compared with urinary elimination. Previous studies have also shown excretion in the feces. Tamura (2) has reported that most of the iodochlorhydroxyquin conjugates are concentrated in the bile and are excreted into the intestinal lumen. A greenish-colored stool has also been seen in experimental dogs suffering from subacute myelo-opticneuropathy (3) and is due to the formation of an iron chelate of the drug.

After topical treatment for 28 d, the plasma levels and urinary excretion of the drug were followed for an additional 12 d. The elimination rate was plotted against time (Fig. 3). The 24-h rate of total elimination decreased from 568 ± 31 μg at 48 h posttreatment to 78 ± 26 μg after 12 d. The plasma concentration fell under the detection limit of our assay method 1 d after discontinuation of treatment. The rate constant of decline of elimination and the apparent half-life ($t_{1/2}$), were calculated from the slope of a plot of the logarithmic rate of elimination against time (Fig. 4) and Eq. 3 (see below). These values are 0.25 ± 0.05 d^{-1} and 3.0 ± 0.5 d, respectively.

The percentage of the drug absorbed was determined by measuring the amount of drug which remained on the back of each dog (14). Sixteen hours after a single 5-g application of 3% iodochlorhydroxyquin cream, 52.5 ± 4.5% of the drug was absorbed. This value is the mean of 10 determinations.

To determine whether the skin was simply acting as a permeable membrane or as a reservoir for the drug, the areas of application on the dogs were again occluded with plastic wrap and gauze without application of the drug, and the plasma levels and urinary elimination were followed for an additional 6

Figure 3—*Mean ± SD of total iodochlorhydroxyquin excreted with time after topical treatment and after again occluding the backs of the dogs with plastic wrap.*
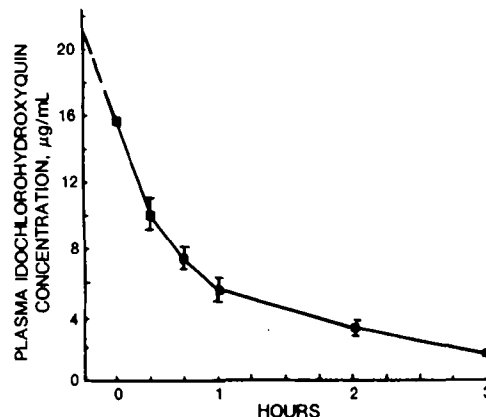

Figure 5—*Nonconjugated plasma concentration plotted with time after intravenous administration of a 100-mg iodochlorhydroxyquin dose in three dogs.*

d. Iodochorhydroxyquin was not detectable in the plasma, whereas the urinary excretion rate increased and, after 3 d of occlusion, again began to decline (Fig. 3). The elimination rate of total iodochlorhydroxyquin was 78 ± 26 μg/24 h at 12 d posttreatment, whereas the rate increased to 153 ± 8 μg/24 h during the third day of occlusion of the application site. These observations suggest that the skin acts as a reservoir for this drug, providing a slow release of I. Occlusion has been previously reported to increase the absorption and penetration of many drugs through the skin due to increased hydration of the stratum corneum and increased skin temperature (14, 15). This mechanism may explain the increase in the elimination of the drug when the site of application was again occluded without drug application.

A single intravenous administration study was conducted to obtain more kinetic data and to observe the effects of placing iodochlorhydroxyquin directly into the systemic circulation. Plasma and urine levels were monitored for 6 d. Three dogs were given 100 mg of I intravenously, and mean plasma concentrations of nonconjugated drug were plotted against time (Fig. 5). The kinetic data were calculated from the slope and intercept of a plot of the log plasma concentration with time (Fig. 6) by:

$$Vd = \frac{dose}{C_0} \qquad (Eq. 1)$$

$$V_c = \frac{dose}{A + B} \qquad (Eq. 2)$$

$$t_{1/2} = \frac{0.693}{k} \qquad (Eq. 3)$$

where Vd is the apparent volume of distribution, V_c is the volume of the central compartment, $t_{1/2}$ is the apparent half-life, C_0 is the initial plasma concentration, A and B are the intercepts from the plot of log C against time, and, k is the apparent rate constant. The data are presented in Table I. The apparent rate constant (β) of the slow elimination phase was calculated from the slope of the plot, whereas apparent rate constant (α) for the rapid decline was calculated by the feathering method (16). A biphasic decline in the plasma levels of I was observed (Fig. 6), which may reflect in part the limited aqueous solubility of the drug (2). Furthermore, the rapid disappearance of the drug from plasma may be due to rapid metabolism as well as rapid tissue distribution as a result of the high lipid solubility of the drug.

Urine samples were also collected at various intervals after intravenous administration of I, and the mean rate of elimination with time is presented in Fig. 7. The urinary elimination of total I is biphasic, with an initial rapid elimination phase followed by a slow elimination phase, reflecting changes seen in plasma levels of the drug. The rate constant for the slow phase was determined from the slope, and the apparent $t_{1/2}$ was calculated by Eq. 3. The values are 0.4 ± 0.1 d^{-1} and 2.0 ± 0.5 d, respectively. The results shown in Figs. 6 and 7 suggest that a rapid distribution of I occurs from blood to tissue,
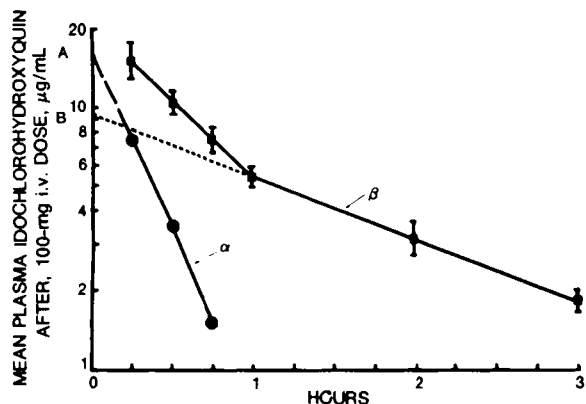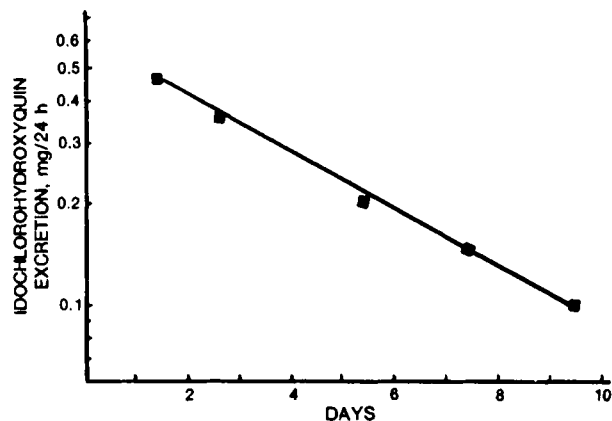
Figure 7—*Excretion rate of total iodochlorhydroxyquin after a 100-mg iv bolus dose.*

followed by a slow elimination of the drug due to the high lipid solubility of iodochlorhydroxyquin.

Liver, kidney, and mesenteric adipose tissue levels of I were analyzed from three dogs. The dogs were killed 2 weeks after intravenous treatment, and the following tissue levels were obtained: liver, 1.22 ± 0.23 μg/g; kidney, 0.83 ± 0.24 μg/g; mesenteric fat, 0.88 ± 0.14 μg/g. In addition, one dog died 15 d after topical treatment of I. The amounts of iodochlorhydroxyquin in the liver, kidney, and mesenteric adipose tissue were 0.74, 0.23, and 0.85 μg/g, respectively. Tissue retention of I has previously been reported. Tamura (2) has found 0.5, 0.3, and 0.1 μg of unconjugated I per gram of fresh liver, mesenteric adipose tissue, and sciatic nerve, respectively, in patients suffering from subacute myelo-opticneuropathy 9 months after oral treatment with I had ceased.

Weight loss occurred in all animals during topical treatment. An average loss of 15.3 ± 2.76% over 28 d was observed, while no weight loss occurred in control animals. Various neurological side effects have been reported after oral administration of iodochlorhydroxyquin (1, 17, 18). No toxicity has been reported after topical application because, in part, long-term studies have not been previously conducted; it has generally been assumed that I is not appreciably absorbed through the skin. During this 28-d study, all dogs became lethargic and less responsive to stimuli. In one of the dogs, partial hind limb paralysis was seen which was identical to that reported by Tateichi and Otsuki (3) after oral administration of iodochlorhydroxyquin. Three of the dogs developed rashes and inflammation of the treated areas. An optic nerve was removed from a dog which was sacrificed after 28 d of topical treatment and examined by electron microscopy. No damage or demyelination was observed. Optic nerve damage after long-term oral use of I has been reported (2). Either the concentrations of I achieved by topical application or the length of time the drug was applied were insufficient to produce optic nerve damage.

One of the dogs died 15 d after topical treatment. The liver was green-gray in color, suggesting hepatocellular toxicity. Microscopic examination of the liver demonstrated diffuse centrilobular and mid-zonal cell necrosis associated with marked dilation and congestion of the sinusoids, as well as the central lobular vein. No significant histological alterations were observed in the kidneys. Three other dogs were sacrificed after completion of the absorption and distribution studies. Histological examination of the kidneys indicated no renal toxicity[4]. However, examination of the livers in each case revealed centrilobular and mid-zonal vacuolization and necrosis with sinusoidal dilation and congestion[4]. The livers of control dogs did not demonstrate these changes. The results suggest that long-term topical application by an occlusion technique, as might be experienced with diaper dermatitis, can result in sufficient absorption to produce hepatotoxicity. Liver and kidney damage after oral treatment is well known and has been previously reported (19, 20).

The achievement of steady-state levels in plasma during topical treatment indicates that accumulation in plasma does not occur. Iodochlorhydroxyquin is rapidly absorbed through the skin. The liver is the main site of metabolism, primarily converting the drug to glucuronide and sulfate conjugates (2). Nonconjugated drug in plasma is transferred to the tissues due to its high lipid solubility (2). As a consequence, prolonged excretion of the drug occurs.

Nonconjugated iodochlorhydroxyquin is neurotoxic (5). Investigations by tissue culture techniques have shown direct toxic effects of the drug on nerves, whereas the glucuronide conjugate produced no cellular toxicity (21).

The results of this study indicate that it is important to use iodochlorhydroxyquin cautiously when topical application is required, particularly when

Figure 6—*Mean concentration ± SD of nonconjugated iodochlorhydroxyquin in plasma after a 100-mg iv bolus injection in three dogs.*

[4] Unpublished results.

it is used for a prolonged period of time and over a large body surface area, due to the extensive percutaneous absorption and the well-recognized toxicity of the drug.

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In Vitro Adsorption–Desorption of Phenethylamines and Phenylimidazoles by a Bentonite and a Resin

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Abstract □ The adsorption of phenethylamines (dextroamphetamine, phentermine, mephentermine, diethylpropion), choline, and phenylimidazoles (levamisole and imazalil) was examined *in vitro* in aqueous solutions on bentonite and on lewatite at 25°C. An ion-exchange mechanism prevails for lewatite and for bentonite up to 0.8 mEq·g⁻¹. The organic cations are more strongly adsorbed on bentonite than on lewatite. On bentonite, the selectivity of adsorption follows the order: primary < secondary < tertiary phenethylamines. An interlamellar monolayer is formed. All drugs, except choline and imazalil, are adsorbed in excess of the cation exchange capacity of bentonite without observable Cl⁻ adsorption and pH changes. Desorption is reversible for lewatite and partially irreversible for bentonite.

Keyphrases □ Phenethylamines—*in vitro* adsorption-desorption by a bentonite and a resin □ Phenylimidazoles—*in vitro* adsorption-desorption by a bentonite and a resin

The prolonged action of drugs in the GI tract with a peak-and-valley pattern of the drug in the blood (1) is realized by binding, *e.g.*, phenethylamines as diethylpropion, to an ion-exchange resin. The same effect was also observed for an oral dose of a mixture of clay-drug compared to the pure drug [*e.g.*, amphetamine, (2)]. Clindamycine, tetracycline (3), and chlorpheniramine (4) are adsorbed by cation exchange under pH conditions favoring the cationic form of the drugs, and they penetrate into the interlayer spaces of montmorillonite, producing an increase in the basal distance.

There are many important clay-organic interactions, *i.e.*, some animal feed additives are adsorbed on vegetable carriers such as maize or soy starch or on resins; bentonites are used as binder and caloric extender for poultry (5); fungicides such as imazalil, used in the foliar treatment of bananas, contaminate the soil. Clay minerals constitute an interesting com-

mercial alternative to resin. Therefore, the adsorption of phenethylamines, choline, a quaternary amine, and two imidazoles on bentonite and lewatite were investigated as model *in vitro* systems. Desorption behavior was tested with simulated gastric and intestinal fluids.

EXPERIMENTAL SECTION

The macroporous (100–200 mesh) sodium lewatite[1] and bentonite[2] were used as adsorbents. Air-dried resin was equilibrated for 2 weeks with an at-
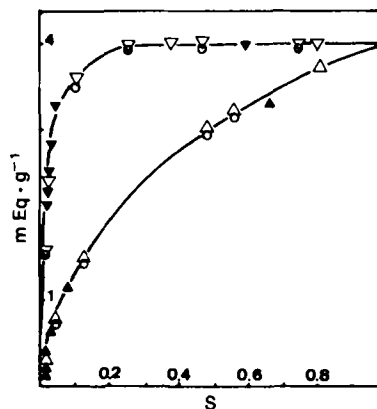

Figure 1—*Adsorption of diethylpropion (△) and imazalil (▽), and sodium desorption (○) on lewatite at pH 4 and 25°C versus S, the equivalent fraction of the organic cation in the equilibrium solution. Filled symbols refer to the desorption of the organic cation with a simulated gastric fluid (0.1 M NaCl, pH 2).*