# *In Vivo* Conversion of $^{14}$C-Labeled Cyclamate to Cyclohexylamine

L. PROSKY and R. G. O'DELL

**Abstract** □ To determine the effects of long-term, low-level feeding of cyclamate on cyclamate metabolism, two groups of weanling rats were fed a chow diet and a chow diet containing 0.1% calcium cyclamate, respectively, for 8 months. Twelve rats from each group were then intubated with $^{14}$C-cyclamate; urine was collected for analysis by a paper electrophoretic method which separates cyclamate, cyclohexylamine, dicyclohexylamine, and *N*-hydroxycyclohexylamine. Elution of the radioactive areas from the paper indicated that none of the controls converted $^{14}$C-cyclamate to additional products, but seven of 11 cyclamate-fed rats converted cyclamate to cyclohexylamine. The labeled cyclohexylamine accounted for 12–25% of the total radioactivity in urine. Two of these urine samples contained traces of radioactive dicyclohexylamine, but *N*-hydroxycyclohexylamine was not found. Isolated liver perfusion studies indicated that the liver is probably not the site of conversion.

**Keyphrases** □ Cyclamate, labeled—conversion to cyclohexylamine and other metabolites, *in vivo*, rats □ Excretion, urinary—$^{14}$C-cyclamate, cyclohexylamine and other metabolites, rats

Until Kojima and Ichibagase (1) reported the presence of cyclohexylamine in urine and feces of one man and one dog ingesting cyclamate orally, the preponderance of evidence indicated a complete and rapid excretion of dietary cyclamate as unchanged cyclamate (2–4). Since that time, several reports supported the presence of metabolic products of cyclamate in urine and feces of man (5–11) and animals (11–13).

The present study was undertaken to ascertain the effects of long-term, low-level feeding of cyclamate on cyclamate metabolism in rats, with special emphasis placed on the separation, identification, and assay of its metabolites in urine. The liver was also studied as a possible site for these conversions.

## EXPERIMENTAL

Male Holtzman weanling rats, weighing 40–60 g., were divided into two groups of 24 animals each and were kept in a room maintained at 24° and 50% humidity. The control group was fed a commercial chow diet[1], and the treated group was fed the chow diet supplemented with 0.1% calcium cyclamate[2] for 8 months. Twelve animals from each group were orally intubated with a known amount (ranging from 2 to 3 μmoles) of an aqueous solution of $^{14}$C-cyclamate[3] (uniformly labeled; specific activity 2.6 μc./μmole); the rats were then placed in individual metabolism cages. Eighteen-hour urine specimens were collected in aluminum foil-wrapped plastic tubes, which were submerged in dry ice during the collection period and kept frozen until analysis. These precautions minimized the conversion of cyclamate to cyclohexylamine in urine specimens.

Metabolites of cyclamate in urine were separated by two TLC systems and one paper electrophoretic system. The urine and appropriate standards (2 μl. of aqueous solutions containing 1 mg./ml. of cyclamate[4], cyclohexylamine[5], dicyclohexylamine[5], or *N*-hydroxycyclohexylamine[5]) were applied to silica gel plates[6]. The plates were developed for 45 min. in acetone–absolute ethanol (1:1), dried in an air stream at room temperature, sprayed with 0.2% 2,7-dichlorofluorescein in methanol, and examined under a UV lamp. The $R_f$ values of the compounds were: cyclamate, 0.71; cyclohexylamine, 0.22; dicyclohexylamine, 0.22; and *N*-hydroxycyclohexylamine, 0.49. A typical chromatogram is illustrated schematically in Fig. 1A.

The second chromatographic system utilized cellulose F plates[7] and a 2.5-hr. developing time in *n*-butanol–acetic acid–water (8:2:1). As shown in Fig. 1B, the metabolic products of cyclamate migrated closer to the solvent front, and the $R_f$ values were: cyclamate, 0.56; cyclohexylamine, 0.73; dicyclohexylamine, 0.94; and *N*-hydroxycyclohexylamine, 0.89. The second method made use of the difference in electrophoretic mobilities of cyclamate and its metabolites in a pyridine–acetic acid–water (8:15:977) system run at 300 mv. for 2 hr. (Fig. 1C). Areas on the paper corresponding to the standards (10 μl. of aqueous solutions containing 1 mg./ml.) were eluted with water and counted for radioactivity in a Nuclear Chicago Mark I scintillation counter[8] using Bray's solution (14). Cyclamate and cyclohexylamine were assayed by previously described colorimetric methods (15, 16).

The liver, suspect as a site of cyclamate metabolism, was perfused in the dark by using the apparatus and procedure described by Green and Miller (17). The livers from three rats known to convert cyclamate to cyclohexylamine were used in conjunction with blood drawn from remaining animals in the group. $^{14}$C-Calcium cyclamate (100 μmoles and 10 μc.), dissolved in 10 ml. of Ringer's solution[9], was infused into the blood supply at a constant rate of 0.16 ml./min. immediately before the blood entered the liver. Aliquots of blood were withdrawn for analysis from the pool formed at the exit from the inferior vena cava at 15-min. intervals for the 2-hr. period of perfusion. At the end of perfusion, the livers were removed from the apparatus, frozen in Freon, and extracted with perchloric acid (18) for later determination of cyclamate and its metabolites.

## RESULTS AND DISCUSSION

In a previous study (19), it was demonstrated that calcium cyclamate at dietary levels not exceeding 1% had little or no effect on a variety of biochemical parameters in the rat. Indeed, others reported that cyclamate fed at a level of 0.43% seemed to stimulate appetite and to cause an increase in body weight (20). In the present study, rats were fed 0.1% cyclamate to eliminate changes in growth or energy metabolism that might be associated with the extremely high doses used by other investigators (12, 21). A working hypothesis was developed which indicated that long-term feeding of cyclamate at low levels would increase its absorption and circulation in the blood and present optimum conditions for cyclamate conversion to cyclohexylamine.

Eighteen-hour urine specimens were collected after intubation of $^{14}$C-cyclamate; previous investigators showed that 70–90% of the radioactivity in cyclamate is excreted in urine and feces in this period (4, 22). Control rats excreted approximately 40% of the radioactive dose in the urine during the 18-hr. collection period

[1] Ralston Purina Co., St. Louis, Mo.
[2] Cyclohexanesulfamic acid calcium salt, Matheson, Coleman & Bell, East Rutherford, N. J.
[3] Mallinckrodt Chemical Works, St. Louis, Mo.
[4] Abbott Laboratories, North Chicago, Ill.
[5] Obtained from the Wisconsin Alumni Research Foundation, Madison, Wis.
[6] TLC plates, silica gel (without fluorescent indicator), Brinkmann Instruments, Inc., Westbury, N. Y.
[7] TLC plates, cellulose F, Brinkmann Instruments, Inc., Westbury, N. Y.
[8] Nuclear Chicago Corp., Des Plaines, Ill.
[9] Cutter Laboratories, Berkeley, Calif.

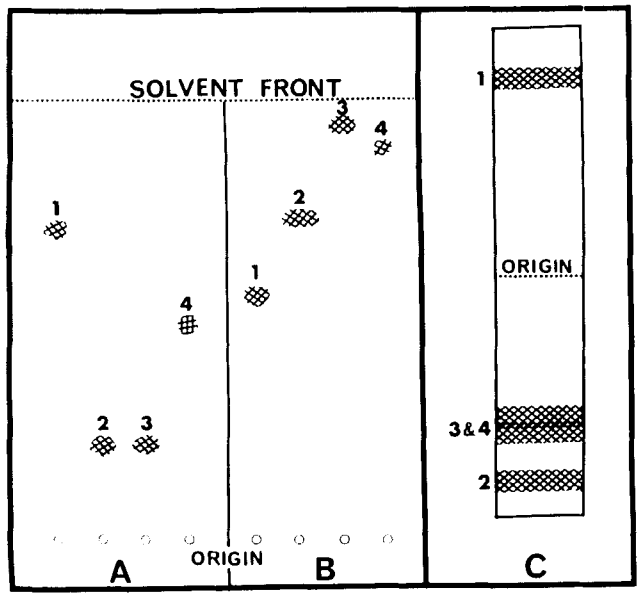


**Figure 1**—*Thin-layer and electrophoretic patterns of cyclamate and its metabolites. Key: A, silica gel plates developed in acetone–absolute ethanol (1:1); B, cellulose F plates developed in butanol–acetic acid–water (8:2:1); and C, paper electrophoresis in pyridine–acetic acid–water (8:15:977), run at 300 mv. The compounds were: 1, cyclamate; 2, cyclohexylamine; 3, dicyclohexylamine; and 4, N-hydroxycyclohexylamine.*

with a range of 22–58%, while treated rats excreted 18–100% during the same period. The wide range of radioactivity excreted in the urine of treated rats was probably related to the size of the cyclamate pool in each rat, *i.e.*, the larger the pool, the smaller the amount of radioactivity in the urine. The erratic excretion of cyclamate, varying from day to day, also tends to spread excretion values.

The distribution of the radioactivity excreted in urine was determined by eluting the areas on paper that corresponded to the authentic compounds after electrophoresis and counting the eluates. As shown in Table I, the radioactivity in the urine of control rats was detected mainly as unchanged $^{14}$C-cyclamate (98.2%). The spot corresponding to cyclohexylamine contained 1.8% of the radioactivity; most of this amount (1.3%) could be attributed to a cyclohexylamine impurity in the original $^{14}$C-cyclamate intubated in the rat. Seven of the 11 treated rats were converters. In the urine of these rats, cyclohexylamine accounted for an average of 18.4% of the radioactivity; the values ranged from 12 to 25%. Two of the seven rats that converted cyclamate to cyclohexylamine also had trace amounts of dicyclohexylamine present in the urine, but this compound accounted for less than 0.1% of the radioactivity excreted. The amount of dicyclohexylamine was too small to assay chemically but could be detected by the dichlorofluorescein spray and was verified by the differential TLC procedures. No *N*-hydroxycyclohexylamine could be detected by either electrophoresis or TLC.

**Table I**—Distribution of Radioactivity Excreted in 18-hr. Urine Collections of Control and Treated Rats Given $^{14}$C-Cyclamate[a]

| Group | Number of Rats | $^{14}$C-Cyclamate, c.p.m. | $^{14}$C-Cyclohexylamine, c.p.m. | $^{14}$C-Cyclohexylamine, %[b] |
|---|---|---|---|---|
| Control (chow) | 12 | 3570 ± 469 | 61 ± 11 | 1.81 ± 0.23 |
| Treated (chow + 0.1% calcium cyclamate) | 7[c] | 5240 ± 1294 | 1209 ± 377 | 18.36 ± 2.00 |

[a] Dosage was a known amount of $^{14}$C-cyclamate containing 2–3 μmoles; specific activity 2.6 μc./μmole. Values are given as means ± *SE*. [b] Calculated as cyclohexylamine ÷ (cyclohexylamine + cyclamate) × 100. [c] Rats that converted cyclamate to cyclohexylamine.

**Table II**—Chemical Determination of Cyclamate and Cyclohexylamine in 18-hr. Urine Collections of Rats[a]

| Group | Number of Rats | Cyclamate, μmoles | Cyclohexylamine, μmoles |
|---|---|---|---|
| Control (chow) | 12 | 0.92 ± 0.08 | 0.02 ± 0.002 |
| Treated (chow + 0.1% calcium cyclamate) | 7[b] | 16.82 ± 5.51 | 3.82 ± 1.63 |

[a] Values are given as means ± *SE*. [b] Rats that converted cyclamate to cyclohexylamine.

The concentrations of cyclamate and cyclohexylamine in the 18-hr. urine specimens, as determined by chemical methods, are shown in Table II. In the case of the treated rats, the specific activity of the intubated $^{14}$C-cyclamate was lowered by the body pool of cold cyclamate and was variable because of different rates of cyclamate absorption and excretion; therefore, excretion of smaller amounts of radioactivity represented larger concentrations of both cyclamate and cyclohexylamine. The percent radioactivity excreted as cyclohexylamine was 10-fold higher for treated rats (Table I), while the actual amount of cyclohexylamine excreted was increased 190 times (Table II). The treated animals, intubated with 2–3 μmoles of $^{14}$C-cyclamate, excreted 16.8 μmoles of cyclamate and 3.8 μmoles of cyclohexylamine, with the difference in amount being supplied by the pool.

Other investigators suggested the intestine as the site of conversion of cyclamate to cyclohexylamine (9, 11, 13). The differences in concentration of cyclohexylamine in urine and feces would indicate that cyclamate is converted to cyclohexylamine in the intestine, with most cyclohexylamine being excreted in the urine; some cyclohexylamine is not absorbed and is excreted in the feces. This type of distribution could also occur if the liver was the site of conversion. The cyclamate could be absorbed from the intestine, converted to cyclohexylamine in the liver, and excreted in urine *via* blood passage through the kidney and in feces *via* the bile. During the isolated perfusion of rat livers of known converters, no metabolites of cyclamate could be detected in blood, liver, or bile; unchanged $^{14}$C-cyclamate was the single radioactive component detected in these tissues. Thus, the liver is probably eliminated as the site of conversion.

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# Polymer–Drug Interaction: Stability of Aqueous Gels Containing Neomycin Sulfate

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**Abstract** □ A method for preparing aqueous gels containing neomycin sulfate is described. Gels of synthetic polymers containing polyionic or polar reactive sites were prepared and stabilized by adsorbing the drug molecule onto a cationic-exchange resin prior to incorporation into the bases. The binding capacity of the drug for the polymers was determined, and the activity of the drug–resin was substantiated and compared relative to an inert gel containing the free drug.

**Keyphrases** □ Neomycin sulfate aqueous gels—preparation, polymer–drug interaction, viscosity and drug activity □ Polymer–drug interaction—neomycin sulfate gels

Neomycin, an epimeric drug molecule, has been shown to interact with numerous macromolecules of biological importance (1–6). Inhibitory activity of the antibiotic toward DNA-dependent RNA polymerase of *Escherichia coli* was shown (1), and formation of a DNA–neomycin complex was found to be a linear function of the DNA concentration (2). Gubernieva and Silaev (3), using heparin as a model, concluded that interaction with basic antibiotics was due to complex formation. They also demonstrated the complex-forming ability of polymyxin, neomycin, and albamycin with plasma proteins, albumin, and γ-globulin in man, rabbits, and rats (4).

Steiner *et al.* (5), using the property of antibiotic complexation, studied the effect of antibiotics on serum cholesterol levels when given orally. When injected, neomycin had no cholesterol-lowering effect. However, when taken orally, the serum cholesterol level was lowered; this suggests a local effect in the GI tract. Oral aureomycin and bonamycin had similar but lesser lowering effects; achromycin, bacitracin, chloromycetin, streptomycin, mycostatin, and penicillin failed to cause any significant lowering of serum cholesterol (6).

Polymeric antibiotics such as neomycin, viomycin, and polymyxin were studied for their interaction with condensed phosphates. The binding capacity of neomycin for these higher phosphates was the strongest (7).

**Table I**—Aqueous Gel Systems

| Polymer | Concentration, % | pH | Gel–Resinate Viscosity, cps. |
|---|---|---|---|
| Methylcellulose 400 cps.[a] | 5 | 7.00 | 36,850 |
| Ethylene maleic anhydride | 4 | 2.55 | 27,410 |
| Carboxyvinyl polymer | 1 | 3.58 | 42,120 |
| Ethylene oxide copolymer | 5 | 7.20 | 35,381 |
| Heteropolysaccharide | 2 | 6.78 | 31,560 |
| Sodium carboxymethylcellulose | 5 | 6.80 | 51,840 |

[a] Reference standard.

Many naturally occurring clays or earths of pharmaceutical importance in liquid or semisolid dosage forms are anionic polyelectrolytes in nature and, therefore, strongly bind medicaments cationic in nature. Physicochemical studies of antibiotic–clay complexes were carried out by Pinck *et al.* (8). They studied the chemical and physical properties of 10 antibiotics; their adsorption by montmorillonite, vermiculite, illite, and kaolinite; and the X-ray diffraction patterns of some of the formed complexes. Streptomycin sulfate, dihydrostreptomycin sulfate, and neomycin sulfate formed complexes to varying degrees with the four clays; X-ray diffraction studies of the complexes indicated monolayer adsorption for strongly basic and dilayer adsorption for amphoteric antibiotics. Danti and Guth (9) studied cation-saturated bentonites and showed that neomycin sulfate was inactivated as a result of an ionic incompatibility with this pharmaceutical agent. Nakashima and Miller (10) qualitatively demonstrated ionic incompatibilities of 18 suspending agents of pharmaceutical importance with several therapeutic agents. Neomycin sulfate showed an immediate precipitating or coagulating action with more than 50% of the agents studied.

A review of the literature, therefore, supports the proposal that neomycin has the greater binding capacity of the antibiotics in common use and presents a more