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Urinary Excretion of Chlorpheniramine and Pseudoephedrine in Humans

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Abstract □ A specific high-pressure liquid chromatographic method for the determination of chlorpheniramine and pseudoephedrine in urine was developed and applied in a urinary excretion study of normal healthy subjects who received a sustained-release dosage form containing 8 mg of chlorpheniramine maleate and 120 mg of pseudoephedrine hydrochloride. Five subjects received one dose on Day 1, followed by multiple dosing every 12 hr for 7 days without ammonium chloride administration. Four subjects received one dose of the sustained-release dosage form together with ammonium chloride. Urine samples were collected during the 1st day and at steady state. The method is specific and simultaneously determines chlorpheniramine, two metabolites (mono- and didesmethylchlorpheniramine), pseudoephedrine, and norpseudoephedrine. The assay recovery was >97% (0.06–3 μg/ml) for chlorpheniramine maleate and >98% (1.5–75 μg/ml) for pseudoephedrine hydrochloride. Excretion of chlorpheniramine and its two metabolites in urine was enhanced after ammonium chloride administration. At steady state, a change in urine pH from 5.69 to 6.46 resulted in more than a 25% decrease in chlorpheniramine and monodesmethylchlorpheniramine excretion. In spite of expected changes in its biological half-life, the overall amount of unchanged pseudoephedrine excreted in urine was not affected by urine pH, presumably because it is primarily excreted in urine as intact drug.

Keyphrases □ Chlorpheniramine—urinary excretion, humans, high-pressure liquid chromatographic analysis □ Chlorpheniramine metabolites—urinary excretion, humans, high-pressure liquid chromatographic analysis □ Pseudoephedrine—urinary excretion, humans, high-pressure liquid chromatographic analysis □ Nasal decongestants—chlorpheniramine and pseudoephedrine, urinary excretion, humans □ High-pressure liquid chromatography—analysis, chlorpheniramine and pseudoephedrine in human urine

Chlorpheniramine and pseudoephedrine, two weak basic drugs, are often used together for treatment of nasal congestion. The extent and rate of urinary excretion of the unchanged drugs are pH and urine flow dependent (1–3). Chlorpheniramine is extensively metabolized *via* *N*-dealkylation and excreted as both mono- and didesmethyl compounds (1, 4–6), and it is expected that the excretion of these metabolites in urine is also urine pH dependent. Pseudoephedrine is primarily excreted unchanged in urine (7).

A GLC method was reported for the determination of chlorpheniramine in urine (2). Similarly, methods utilizing GLC (8, 9) or a radiolabeled technique (3) were reported for the determination of pseudoephedrine in urine. The purposes of this investigation were: (*a*) to develop a simple, specific, high-pressure liquid chromatographic (HPLC) method for the simultaneous determination of chlorpheniramine, its demethylated metabolites, and pseudoephedrine and (*b*) to apply this method in studying the urinary excretion of these drugs and their metabolites in humans.

EXPERIMENTAL

Protocol—Nine healthy, nonobese, male subjects participated. They did not receive any drugs, including enzyme-inducing agents and monoamine oxidase inhibitors, 1 month before and during the study. Caffeine-containing and alcoholic beverages also were withheld during the entire study.

Five of the subjects received one sustained-release capsule[1] containing 8 mg of chlorpheniramine maleate and 120 mg of pseudoephedrine hydrochloride on Day 1, after an overnight fast, followed by an additional 4-hr fasting period. Thereafter, the subjects received one capsule every 12 hr for 6 days (Days 2–7) and then one capsule on Day 8. Cumulative urine samples were collected during the 0–24-hr period and at the steady state during the 156–168- and 168–180-hr periods.

The other four subjects received one single capsule with and without ammonium chloride treatment according to a double crossover design. A washout period of 1 week was allowed between treatments. The capsule was always taken after an overnight fast and followed by 4 hr of fasting. One day before and throughout the study period (~68 hr), two enteric coated ammonium chloride tablets[2] (500 mg each) were administered every 2 hr. The first dose taken before retiring and that given on arising always consisted of four tablets (2 g). When a subject's urinary pH was >5.3, one additional ammonium chloride tablet was administered after the urinary pH measurement. If the urinary pH was <4.9, the next scheduled dose was omitted. An attempt was made to control the urine flow by frequent oral administration of liquids throughout the study periods. Urine samples were collected from −1 to 0 hr, *i.e.*, just before the drug was administered, and at frequent intervals up to 48 hr.

During the entire investigation, standard meals were given to all subjects. Urine pH was determined at room temperature, and the volume of each urine sample was measured in a graduated cylinder. An aliquot of 30 ml of the thoroughly mixed sample was withdrawn and stored at −15° until assayed.

Assay—Chlorpheniramine and pseudoephedrine concentrations were determined according to the following methods.

Method I—Two-milliliter samples of urine or standard solution containing 0.06–3 μg of chlorpheniramine maleate[3]/ml and 1.5–75 μg of pseudoephedrine hydrochloride[4]/ml were taken for assay. To the samples were added 0.5 ml of 5% KOH solution and 5 ml of an extraction solvent consisting of hexane–methylene chloride (60:40). The samples were shaken for 30 min and centrifuged. Four milliliters of the solvent was pipetted into a vial containing 0.5 ml of chlorpromazine hydrochloride[5], 1 μg/ml in methanol. The mixture was evaporated under nitrogen. The residue was dissolved in 50 μl of methanol from which 20 μl was removed for injection.

Method II—Two-milliliter samples of urine or standard solution

[1] Arnar-Stone Laboratories, McGaw Park, Ill.
[2] Eli Lilly & Co., Indianapolis, Ind.
[3] E. M. Labs, Elmsford, N.Y.
[4] A. G. Knoll, Whippany, N.J.
[5] H. Reisman Corp., Orange, N.J.

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Table I—Recovery and Day-to-Day Variation of the Simultaneous HPLC Determination of Chlorpheniramine and Pseudoephedrine in Urine

Concentration Range, μg/ml	Mean Percent Recovery ± SD		
		Method II[a]	
	Method I	First Determination	Second Determination
Chlorpheniramine			
0.06–3	97.3 ± 4.47	102.8 ± 1.61	97.9 ± 3.84
n	30	23	8
Pseudoephedrine			
1.5–75	89.7 ± 11.2	98.1 ± 4.78	98.4 ± 7.16
n	30	23	8

[a] There were 3 days between the first and second determinations.

containing 0.06–3 μg of chlorpheniramine maleate/ml and 1.5–75 μg of pseudoephedrine hydrochloride/ml were taken for the assay. To the samples were added 0.5 ml of 5% KOH solution and 4 ml of an extraction solvent consisting of ether–methylene chloride (70:30). The samples were shaken for 30 min, centrifuged, and then immersed in a dry ice–isopropanol bath.

After the aqueous layer was frozen, the organic layer was transferred to a clean tube containing 100 μl of imipramine hydrochloride[6] solution (8 μg/ml in 0.5% phosphoric acid). The mixture was vortexed, centrifuged,

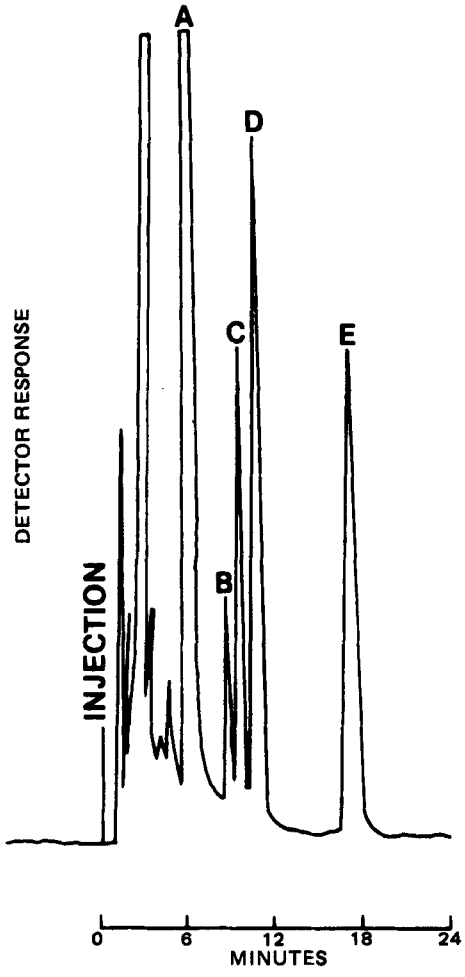

Figure 1—*Typical chromatogram of a urine sample from a subject after multiple-dose administration (for 8 days every 12 hr) of the sustained-release capsule containing 8 mg of chlorpheniramine maleate and 120 mg of pseudoephedrine hydrochloride. This sample was assayed according to Method I. Key: A, pseudoephedrine at 6 min; B, didesmethylchlorpheniramine at 8.5 min; C, monodesmethylchlorpheniramine at 9.0 min; D, chlorpheniramine at 10 min; and E, internal standard (chlorpromazine) at 16.0 min.*

[6] Sigma Chemical Co., St. Louis, Mo.

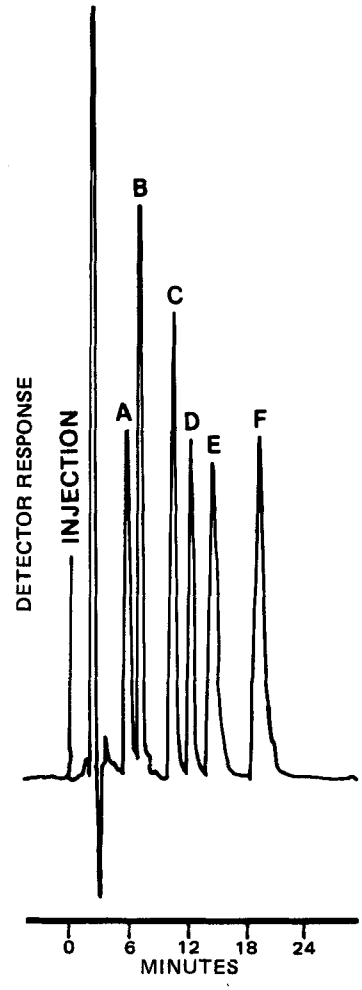

Figure 2—*Typical chromatogram of a blank urine sample spiked with the mixture of chlorpheniramine, pseudoephedrine, and their metabolites. This sample was assayed according to Method II. Key: A, norpseudoephedrine at 6.30 min; B, pseudoephedrine at 7.20 min; C, didesmethylchlorpheniramine at 10.6 min; D, monodesmethylchlorpheniramine at 12.3 min; E, chlorpheniramine at 14.6 min; and F, internal standard (imipramine) at 19.5 min.*

and frozen again. The organic layer was discarded and the aqueous layer was purified of residual organic solvents under vacuum. Twenty microliters of the remaining solution was injected.

For both methods, all glassware was washed first with 4 *N* nitric acid followed by a methanol wash. The specificity of each method was tested for two chlorpheniramine metabolites, mono- and didesmethylchlorpheniramine[7]. Method II also was tested for norpseudoephedrine[8].

All urine and the standard solution samples were assayed with a high-pressure liquid chromatograph[9] fitted with one[10] (Method I) or two[11] (Method II) 30-cm × 4-mm i.d. columns. The columns were packed with microparticulate silica permanently coated with a propylcyano layer. The mercury source UV absorbance detector was equipped with a 254-nm filter[12]. The flow rate of the mobile phase, acetonitrile–methanol–phosphate buffer (0.005 *M* Na_2HPO_4 and 0.01 *M* NaH_2PO_4, pH 6.6) (25:25:50), was 2 ml/min. The final pH of the mobile phase was 7.6.

RESULTS

Figure 1 depicts a typical chromatogram of a urine sample from a subject who received multiple dosing of the capsule containing 8 mg of

[7] Courtesy of Smith Kline & French Laboratories, Philadelphia, Pa.
[8] Aldrich Chemical Co., Milwaukee, Wis.
[9] Model ALC/GPC 204, Waters Associates, Milford, Mass.
[10] μBondapak CN column, packed in hexane, manufactured in 1977 and marketed until August 1977; Waters Associates, Milford, Mass.
[11] μBondapak CN column, packed in methanol–water (70:30), introduced in the market in December 1977; Waters Associates, Milford, Mass.
[12] Model 440 detector, Waters Associates, Milford, Mass.

Table II—Effect of pH on Excretion of Unchanged Chlorpheniramine and Pseudoephedrine in Urine

Urinary Excretion Time, hr	Urine pH	Total Amount Excreted[a], Mean ± *SD*, mg			
		Chlorpheniramine	Monodesmethyl-chlorpheniramine	Didesmethyl-chlorpheniramine	Pseudoephedrine
0–24 (single dose)[b]	5.31 ± 0.315	1.19 ± 0.267	0.835 ± 0.242	0.284 ± 0.158	116 ± 6.78
0–24 (single dose)[b]	5.95 ± 0.407	0.438 ± 0.071	0.537 ± 0.173	0.161 ± 0.163	104 ± 8.52
0–24 (multiple dose)	6.16 ± 0.470	0.557 ± 0.595	0.767 ± 0.517	0.265 ± 0.124	104 ± 10.3
156–168 (steady state)	5.69 ± 0.434	1.44 ± 1.19	1.80 ± 0.558	0.373 ± 0.086	109 ± 22.5
168–180 (steady state)[c]	6.46 ± 0.507	1.07 ± 1.26	1.32 ± 0.428	0.366 ± 0.119	104 ± 21.5

[a] These values are expressed in equivalents of chlorpheniramine maleate (for chlorpheniramine and its metabolites) and pseudoephredrine hydrochloride. [b] Mean of four subjects; all others are means of five subjects. These subjects received the drugs with and without ammonium chloride in a two-way crossover fashion. [c] Statistically significant difference (paired *t*-test) from 156–168-hr period with respect to urine pH ($p < 0.005$), chlorpheniramine ($p < 0.02$), and monodesmethylchlorpheniramine ($p < 0.025$).

chlorpheniramine maleate and 120 mg of pseudoephedrine hydrochloride. Figure 2 shows a typical chromatogram of a blank urine sample spiked with chlorpheniramine, mono- and didesmethylchlorpheniramine, pseudoephedrine, and norpseudoephedrine. The chromatograms shown in Figs. 1 and 2 were determined by assay Methods I and II, respectively.

Table I summarizes the results of the recovery and reproducibility study for chlorpheniramine and pseudoephedrine by both methods. The recovery with Method I was 97.3 ± 4.47% (mean ± *SD*, $n = 8$) for chlorpheniramine and 89.7 ± 11.2% for pseudoephedrine. The recovery with Method II, using the same urine samples 3 days later, was 97.9 ± 3.84% for chlorpheniramine and 98.4 ± 7.16% ($n = 8$) for pseudoephedrine. There was no correlation between the recovery and the urine concentration for either drug.

Figure 3 depicts the day-to-day reproducibility of Method II. A plot of the determined concentrations on the 1st day (x) *versus* the corresponding concentrations of the same urine samples determined 3 days later (y) gave regression lines of $y = 0.079 \pm 0.980x$ ($r = 0.999$) for 0.06–3 μg of chlorpheniramine maleate/ml of urine and $y = 0.0637 \pm 0.961x$ ($r = 0.997$) for 1.5–75 μg of pseudoephedrine hydrochloride/ml of urine.

Figure 4 shows the average time course of cumulative urinary excretion of chlorpheniramine and its metabolites in three subjects after ingestion of a single capsule containing 8 mg of chlorpheniramine maleate and 120 mg of pseudoephedrine hydrochloride. Table II summarizes the amounts of chlorpheniramine, its two metabolites, and pseudoephedrine excreted unchanged in urine after a single dose and at steady state after administration of the same dose every 12 hr for 8 days. Of the chlorpheniramine dose, 18.0% was excreted intact, 22.5% was excreted as monodesmethylchlorpheniramine, and 4.7% was excreted as didesmethylchlorpheniramine. During the same period, 91% of the pseudoephedrine dose was excreted intact in this group of subjects.

Table II also shows that a significant elevation in urine pH from an average of 5.69 to 6.46 apparently caused a significant decrease, about 25%, in the amount of chlorpheniramine and monodesmethylchlorpheniramine excreted in urine. There was no such effect on the excretion of pseudoephedrine in urine at steady state. Ammonium chloride enhanced excretion of chlorpheniramine and its metabolites but had relatively little effect on the total unchanged pseudoephedrine excreted in urine (Table II). The amount of norpseudoephedrine excreted in urine of these subjects was negligible with or without ammonium chloride treatment.

DISCUSSION

Method I was originally developed for a simultaneous determination of chlorpheniramine and pseudoephedrine in urine using a propylcyano HPLC column. It was successfully utilized to assay the urine samples from the multiple-dose study as well as some samples from the single-dose study. During an interim period, the HPLC column was improved by the manufacturer. Because of this change, certain adjustments had to be made in the extraction procedure, resulting in the development of Method II. Additionally, the internal standard from Method I, *i.e.*, chlorpromazine, was replaced by imipramine, which showed better stability under the acidic conditions of Method II and had a shorter retention time.

The assay is specific for the simultaneous determination of unchanged chlorpheniramine and pseudoephedrine. It also separates two metabolites of chlorpheniramine, mono- and didesmethylchlorpheniramine, and the pseudoephedrine metabolite, norpseudoephedrine. In spite of the similarity in the HPLC mobile phases, the retention times of the individual compounds determined by Method II were relatively longer than those determined by Method I. Method II also provided better resolution than did Method I. This finding was expected since the new assay utilized two columns and, therefore, the number of the theoretical chromatographic plates was larger, which consequently resulted in improved resolutions of the compounds by HPLC.

Both chlorpheniramine and pseudoephedrine are stable in aqueous solution under drastic temperature conditions (10). There was no indication of any instability in urine. The reproducibility and precision of the assay are very high for both compounds, as evidenced by relatively small coefficients of variation, 1.6 and 4.8% for chlorpheniramine and pseudoephedrine, respectively, and very little day-to-day variation (Table I and Fig. 3).

Chlorpheniramine is extensively metabolized to mono- and didesmethylchlorpheniramine and other nonextractable metabolites (4). In this study, under steady-state conditions, only about one-half of the dose was excreted in urine as chlorpheniramine and its two metabolites within one dosing interval. This result suggests that chlorpheniramine is excreted as unextractable metabolites in urine since there was no evidence of other measurable compounds by HPLC. Consistent with theory and the published literature (2), excretion of unchanged chlorpheniramine was urine pH dependent. The excretion of the two demethylated chlorpheniramine metabolites was also pH dependent after a single dose and at steady state. A small fluctuation in the urine pH at steady state

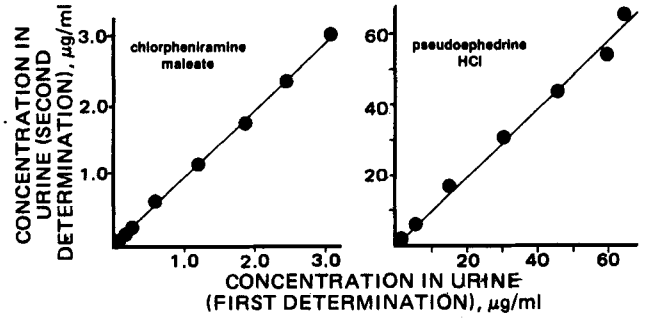

Figure 3—*Day-to-day variation of the simultaneous HPLC determination of chlorpheniramine and pseudoephedrine in urine by Method II. There were 3 days between the first and second determinations.*

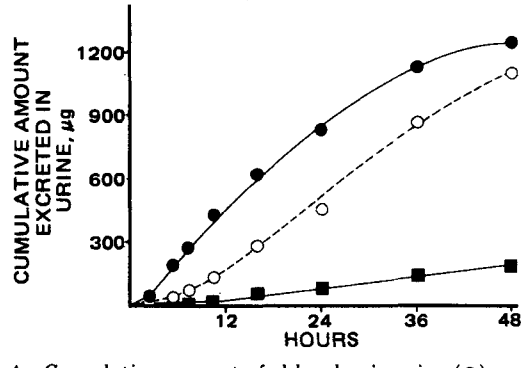

Figure 4—*Cumulative amount of chlorpheniramine (●) and its metabolites, monodesmethylchlorpheniramine (○) and didesmethylchlorpheniramine (■), expressed as chlorpheniramine equivalents following administration of a single dose of 8 mg of chlorpheniramine maleate and 120 mg of pseudoephedrine hydrochloride in a capsule to three normal subjects who also received ammonium chloride throughout the study.*

caused substantial changes in excretion of chlorpheniramine and the monodesmethyl metabolite. In this study, the change in urine pH during two different urine collection intervals was expected and was presumably due to variations in diet.

The urinary pseudoephedrine excretion rate was enhanced under controlled acidic urine pH (3). However, the overall amount excreted unchanged was not dependent on urine pH. The excretion of negligible norpseudoephedrine, the principal metabolite, with and without pH control also supports this observation.

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Surface Properties of Membrane Systems: Interaction of Ketamine with Monomolecular Films of Gangliosides and Mitochondrial Lipids

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Abstract □ Ketamine solutions did not form a film ($\pi = 0$) but had an appreciable surface potential (ΔV = 500 mv), indicating a significant array of ± oriented charge dipoles at the air–water interface, as opposed to calcium chloride solutions whose ΔV was zero. The ΔV values of ganglioside films spread on the aqueous phase varied in the order water < sodium chloride < calcium chloride < ketamine hydrochloride. At equivalent concentrations, calcium chloride was 500 times as effective as sodium chloride, and ketamine at the clinical concentrations of 10–20 μg/ml (36–72 μ*M*) was 6000 times as effective as calcium chloride in raising the surface potential of gangliosides; the ΔV effect with mitochondrial lipid was in the reverse order: water < sodium chloride = ketamine hydrochloride < calcium chloride. This calcium–ketamine inversion indicates a unique specificity of ketamine for gangliosides. Since ketamine acts on the brain and did not affect mitochondrial respiration, the surface potential data suggest that part of the mechanism of action of ketamine could be its interaction with synaptic surfaces and, specifically, with the sialic acid of gangliosides and/or glycoproteins present on the synaptic membrane surface.

Keyphrases □ Membranes—surface properties, ketamine interaction with ganglioside and mitochondrial lipid monomolecular films □ Ketamine—effect on monomolecular films of gangliosides and mitochondrial lipids □ Gangliosides—monomolecular films, effect of ketamine □ Mitochondrial lipids—monomolecular films, effect of ketamine □ Analgesics—ketamine, effect on monomolecular films of gangliosides and mitochondrial lipids

Ketamine, a potent analgesic, acts on the cerebral cortex (1–5). Although the pharmacological effects of ketamine on the cardiovascular and respiratory systems have been defined clinically and experimentally (1–4), little is known about its mechanism of action at the molecular level. A relatively small molecule, which is prepared synthetically as the 2-(*o*-chlorophenyl)-2-(methylamine)cyclohexanone hydrochloride (mol. wt. 275), ketamine has been widely used and discussed as an analgesic, a dissociative anesthetic, and a preanesthetic in experimental and clinical surgery (1–4).

Although rapid induction of analgesia and cardiovascular stimulation with little effect on respiration and respiratory resistance are recognized effects of ketamine (1–4), it is believed that the major effects are a consequence of the direct action of ketamine on the central nervous system (1–4), specifically, the cerebral cortex (5). As a working hypothesis, it was assumed that the negatively charged surfaces of the synaptic membranes in the cerebral cortex are the target of this positively charged molecule.

The surface potential of the monomolecular film at the air–water interface is a suitable model in which to study the ionic properties of both the ketamine and the acidic synaptic components. Interaction of positively charged ketamine with negatively charged lipid films was expected to increase the surface potential (6, 7), which could then be related to film pressure and ion concentrations in the aqueous phase. Because of the role that Ca^{2+} plays in bioelectric phenomena and in the release of acetylcholine from synapses (5), the effects of Ca^{2+} and Na^{+} ions in the subphase were studied also.

The acidic lipid of choice was ganglioside; it is abundant in the synapses in the gray matter forming the cerebral cortex (8). For a comparison, a neutral phospholipid, dipalmitoyl phosphatidylcholine (dipalmitoyl lecithin), was used since the phosphatidylcholines (lecithins) are important constituents of cell membranes. The total lipid extract of mitochondria and isolated cardiolipin were studied for two reasons: (*a*) the mitochondrion is an important intracellular organelle, which controls vital biochemical processes; and (*b*) besides lecithin, the mitochondrial lipid contains about 10% cardiolipin (diphosphatidylglycerol), which is also acidic but is quite different from the ganglioside.

EXPERIMENTAL

Highly purified ketamine[1] and reagent grade electrolytes were used.

[1] Gift of Dr. A. M. Moore, Parke-Davis and Co., Ann Arbor, Mich.

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