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Article *in* Journal of Pharmacology and Experimental Therapeutics · June 1983

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THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 225, No. 2
Printed in U.S.A.

Formation of Methylamines from Ingested Choline and Lecithin[1]

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Accepted for publication February 3, 1983

ABSTRACT

Humans ingest substantial amounts of choline and lecithin as part of common foods. Physicians have recently begun administering large doses of these compounds to individuals with neurological diseases. A significant fraction of ingested choline is destroyed by enzymes within gut bacteria, forming trimethylamine (TMA), dimethylamine (DMA) and monomethylamine (MMA). Some of these methylamines are eventually excreted into the urine, presumably after being absorbed and carried to the kidneys *via* the bloodstream. The methylamines formed after choline is eaten could be substrates for the formation of nitrosamines, which have marked carcinogenic activity. Twenty-seven millimoles of choline chloride, choline stearate or lecithin were administered to healthy human subjects. It was found that these treatments markedly increased the urinary excretion of TMA, DMA and MMA, with choline chloride having the greatest effect. Rats were treated with 2 mmol/kg b.wt. of choline chloride or lecithin, and it was found that these treatments significantly increased urinary TMA excretion and did not alter DMA or MMA excretion. Our choline chloride preparation contained no MMA, DMA or TMA; however, it was found that our choline stearate and all the commercially available lecithins tested were contaminated with methylamines. Prior removal of methylamines from our lecithin preparation minimized the effect of oral administration of this compound on methylamine excretion in urine of rats and humans.

The ingestion of choline or lecithin results in increased blood and brain choline concentrations and in increased synthesis and release of acetylcholine by neurons (Haubrich, *et al.*, 1975; Cohen and Wurtman 1975, 1976). Physicians have recently been administering choline (5–10 g/day) or lecithin (20–30 g/day) orally for the treatment of neurological diseases. In several clinical trials (including some with double-blind protocols) such treatments have been effective in reducing the symptoms of tardive dyskinesia, a movement disorder which apparently arises as a side effect of antipsychotic medications (Davis *et al.*, 1975; Gelenberg *et al.*, 1979; Growdon *et al.*, 1977; Jackson *et al.*, 1979). Choline and lecithin are currently also being used for, or being tested as, treatments for Tourette's syndrome (Barbeau, 1980; Stahl and Berger, 1980), memory disorders (Boyd *et al.*, 1977; Fovall *et al.*, 1980; Sitaram *et al.*, 1978; Zeisel *et al.*, 1981), mania (Cohen *et al.*, 1980) and ataxia (Lawrence *et al.*, 1980; Legg, 1978). At the present time many humans are partaking in chronic therapy with large amounts of choline-containing compounds as part of the legitimate scientific studies outlined above. Choline and lecithin are also available without prescription in "health food" stores. No accurate estimates of such sales are available, but we assume that they are significant.

Choline is found in many common foodstuffs (Zeisel, 1981), often in the form of the choline-containing phospholipid lecithin. We have estimated that humans in the U.S.A. ingest between 300 and 1000 mg of choline per day (Zeisel *et al.*, 1982). Lecithin is a component of all biomembranes; thus most foods contain some. Meat, for example, contains approximately 0.5% lecithin (Pensabene *et al.*, 1975). In addition, each adult in the U.S.A. consumes approximately 100 mg/day of lecithin that has been added to foods by manufacturers who use it as an emulsifier (Select Committee on GRAS Substances, 1979).

A significant, but unknown, fraction of ingested choline is destroyed by enzymes within gut bacteria, forming TMA, DMA and MMA (Asatoor and Simenhoff, 1965). Some of these methylamines are eventually excreted in the urine, presumably after being absorbed and carried to the kidneys by the bloodstream. [The TMA excreted in this manner is responsible for the "fishy" body odor noted in humans ingesting large amounts of choline (Growdon *et al.*, 1977).] Choline is needed by all cells for incorporation into membrane lecithin and for the synthesis of acetylcholine by neurons (Zeisel, 1981). The metabolism of choline by gut bacteria wastes this nutrient, at the same time that it exposes humans to potentially harmful methylamines. Ingested lecithin is deacylated by pancreatic phospholipase and then is absorbed as lysolecithin by gut mucosal cells. Thus, little free choline is generated within the intestinal lumen (Zeisel, 1981). For this reason, it is held that little methylamine

Received for publication April 6, 1982.
[1] This work was supported in part by Grant 1-P01-CA26731-01 from the National Institutes of Health.
[2] A John A. and George L. Hartford fellow of the John A. Hartford Foundation.

ABBREVIATIONS: TMA, trimethylamine; DMA, dimethylamine; MMA, monomethylamine.

should be generated from dietary lecithin, assuming that such lecithin is not itself contaminated with methylamines or free choline.

We now describe the effects of orally administered choline and lecithin upon the excretion of methylamines in urine by humans and rats. We also present data which indicate that commercially available lecithins are contaminated with methylamines.

Materials and Methods

Animals. Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were housed in metabolic cages, in a controlled environment (24°C; 12 hr of light from 6:00 A.M. to 6:00 P.M.). Animals were fed rat chow and water *ad libitum* except as noted.

Materials. The synthetic diets for rats (choline-free, low-choline and high-choline) consisted of 250 g of casein, 500 g of sucrose, 100 g of dextrin, 40 g of Rogers-Harper mineral mix, 22 g of choline-free vitamins (ICN Corporation, Cleveland, OH), 35 g of agar dissolved in 1 liter of water and 250 g of corn oil (Mazola). Choline chloride (Anachemia, Montreal, Canada) was added as indicated. Choline stearate was obtained from Orgamol S.A. (Evionnaz, Switzerland). Lecithins used were as follows: Bolec Soy and Egg (Unilever Corporation, Vlaardingen, Netherlands); Phospholipon 100 and Nattermann Egg (Nattermann, Koln, Germany); Alcolec 640 and Alcolec granules (American Lecithin, Woodside, NY); Leci-Fiber (MLO Products, Hayward, CA); Formula 92 granules (Plus Products, Irvine, CA); Lecitol (Hoffman Products, York, PA); Three Star Soya (Three Star Company, Los Angeles, CA); Liquid Lecithin (Fearn Soya Foods, Melrose Park, IL); Schiff Soy Lecithin (Schiff Bio-Food Products, Moonachie, NJ); Food-Tritional Soya granules (Vogue Nutritional Products, North Billerica, MA); Golden Cal Powder (Golden California Company, Chatsworth, CA); and Pharmacaps capsules (Pharmacaps Incorporated, Elizabeth, NJ).

Preparation of "clean" lecithin. Bolec Soy Lecithin was dissolved in ethanol and water was then added. After mixing, acetone was added to precipitate the lecithin. The supernatant was decanted, and the precipitate was washed two times with equal volumes of water. Lecithin prepared in this manner contained only 4% as much TMA (0.6 μmol/g) as the untreated compound (see table 1).

Collection and preparation of samples. Urine was collected into a jar containing 50 ml of toluene (as an antibacterial agent) and 50 ml 2N HCl in human studies, and was collected into a 30-ml tube containing 5 ml of toluene and 0.5 ml 2 N HCl in the rat studies. Samples of urine were acidified (final solution was approximately 0.1 N HCl) so as to form the methylamine hydrochlorides which are considerably less volatile than the parent amines. The acidified urines were centrifuged at 3000 rpm for 10 min at 4°C, and 1 ml of the supernatant was placed in a sealed vial which had a Teflon-lined diaphragm in the cap. One ml of 1 N NaOH was added to the 1 ml of urine in the vial. After the addition of NaOH to the sealed vials, samples were stable for several days at 4°C. One microliter of this mixture was injected into a gas chromatograph column.

Samples of commercial lecithins were dissolved in chloroform; water and KCl were added and the solution mixed. After centrifugation at 10,000 × *g* for 10 min, the aqueous phase was saved and acidified so that it was 0.1 N HCl, and was then prepared for injection into the gas chromatograph.

Gas chromatograph method for the determination of methylamines. MMA, DMA and TMA were determined using a procedure modified from Di Corcia and Samperi (1974). Chromatography was performed using a Packard GC-430 equipped with a flame ionization detector. We used Carbopack B/4% CW 20M/0.8% KOH packing (Supelco) in a 6 foot (length) × 2 mm (inside diameter) glass column. The injection port was glass lined because methylamines are adsorbed to metal; it was heated to 250°C. The flow of nitrogen carrier gas was 20 ml/min. Initial oven temperature was 60°C for 4 min, then increased at a rate of 30°C/min to a final temperature of 220°C, which was maintained for 0.5 min. Detector temperature was 250°C. Amines were quantitated using the electronic integration of the Packard GC-430. An internal standard of propylamine was used to correct for variation in injection volume.

TABLE 1

Methylamine contaminants in preparations of choline-containing compounds used to treat humans

Choline chloride was dissolved in water and 1 μmol was injected onto the gas chromatograph. Choline stearate and lecithins were dissolved in chloroform and water was then added. An aliquot of the aqueous phase was then acidified and prepared for injection onto the gas chromatograph using the methods described in the text. Lecithin purity was determined using an assay for choline which is described under "Materials and Methods."

Preparation	Amine		Lecithin
	DMA	TMA	
	μmol/g preparation		mg/g preparation
Choline chloride	ND*	ND	
Choline stearate	ND	500.00	
Soy Lecithin (Bolec-soup)	0.75	11.95	857
Soy Lecithin (Bolec, produced 1979)	ND	14.62	796
Soy Lecithin (Bolec, produced 1980)	ND	11.68	858
Egg Lecithin (Bolec, produced 1979)	0.55	6.83	597
Soy Lecithin (Phospholipon 100)	ND	5.38	687
Egg Lecithin (Nattermann, produced 1978)	0.12	2.86	223
Soy Lecithin (Alcolec 640)	ND	2.84	258
Soy Lecithin (Alcolec granules)	ND	1.47	142
Soy Lecithin (Pharmacaps capsules)	ND	1.33	190
Soy Lecithin (Food-Tritional)	ND	0.78	162
Soy Lecithin (Formula 92 granules)	ND	0.76	163
Soy Lecithin (Fearn Liquid)	ND	0.87	97
Soy Lecithin (Schiff)	ND	0.93	152
Soy Lecithin (Three Star Soya)	ND	0.94	140
Soy Lecithin (Lecitol)	1.26	4.51	69
Soy Lecithin (Leci-Fiber)	1.78	1.57	47
Soy Lecithin (Golden Cal Powder)	1.68	4.20	68

* ND, not detected.

Identification of methylamines using the mass spectrometer. Mass spectra were obtained on a Hewlett-Packard gas chromatography-mass spectometer, model 5992A, equipped with a glass column [6 foot (length) × 2 mm (inside diameter)] packed with 4% CW 20M/0.8% KOH on Carbowax B (the same packing as used for the quantitation of methylamines using the flame ionization detector on the Packard gas chromatograph). Analyses were carried out at 60°C. Carrier gas (He) flow rate was adjusted for optimal performance of the jet separator (approximately 20 ml/min).

Two urine samples and two lecithin samples which had been previously analyzed using the Packard gas chromatograph and flame ionization detector were prepared for injection onto a gas chromatograph as described earlier. Components with retention times and mass spectra indistinguishable from those of authentic DMA and TMA were identified. The mass spectra, in addition, contained no extraneous ions, indicating that each of the chromatographic peaks represented only a single major component.

Lecithin determination. Lecithin was isolated using thin-layer chromatography (Whatman LK5D silica gel plates eluted with chloroform-methanol-water, 65:30:4, v/v) and was then hydrolyzed using 6 N HCl at 80°C for 1 hr. The resulting free choline was then assayed as described below.

Choline determination. Choline was isolated and measured using liquid cation exchange chromatography and a radioenzymatic assay in which choline kinase was used to catalyze the formation of radiolabeled phosphorylcholine (Goldberg and McCaman, 1973).

Procedure. Healthy, normal human volunteers gave informed consent for participation in these studies. None of the subjects was in the habit of smoking cigarettes. Subjects consumed equimolar doses of one of the test compounds before their breakfast meal (3.5 g of choline

chloride in orange juice, 10.2 g of choline stearate in orange juice, 20.0 g of lecithin (Bolec Soy) in chicken soup, 120 ml of orange juice or 300 ml of chicken soup). During the study all subjects ate their normal meals. On each study day subjects collected their urine, which was analyzed for methylamines as described earlier.

Rats were place on a choline-free diet for 48 hr. All urine was collected during the second 24-hr period. During hour 49, animals were treated with a single orogastric feeding of choline chloride or lecithin (Bolec Soy). These doses delivered 200 mg of free choline base per kg b.wt. The control group was intubated with 2 ml of 0.9% NaCl (saline). The rats continued to ingest the choline-free diet, and urine was collected for another 24-hr period. Aliquots of urine were treated as described under "Materials and Methods" and were assayed for methylamines.

Statistics. Data were analyzed using the Student's *t* test and one-way analysis of variance.

Results

The gas chromatographic separation of methylamines was excellent (fig. 1). We chose to use the minimal amount of manipulation of biological samples before injection onto the gas chromatograph. This reduced the loss of the volatile amines, and yet did not sacrifice base-line separation of our peaks. The properties of these amines were such that few other compounds had similar elution times, even when complex biological samples such as urine were assayed. We tested many compounds in our system, and we found that only ethylamine eluted near the peaks of interest. Ethylamine formed a small shoulder on DMA peaks (fig. 1). We always injected a water blank between experimental samples. This technique, along with presoaking our glass injection port in KOH, minimized problems due to "ghosting" (retention of amines due to binding to acidic sites on the injection port, with release during subsequent injections). This assay was capable of detecting as little as 100 pmol or as much as 10 nmol of amine. We were able to confirm the identity of the amines within our peaks as DMA and TMA by using mass spectrometric analysis.

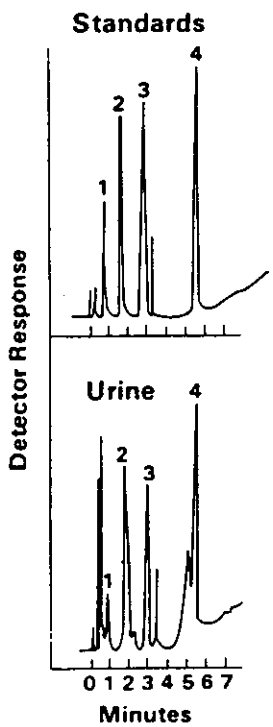

Fig. 1. Separation of methylamines using the gas chromatograph. Samples of standard solutions and urine were prepared as described under "Materials and Methods." Chromatography was performed using a Packard GC-430 equipped with a flame ionization detector. We used Carbopack B/4% CW 20M/0.8% KOH packing (Supelco) in a 6 foot (length) × 2 mm (inside diameter) glass column. The flow of nitrogen carrier gas was 20 ml/min. Initial oven temperature was 60°C for 4 min, then increased at a rate of 30°C/min to a final temperature of 220°C, which was maintained for 0.5 min. Detector temperature was 250°C. Typical chromatograms of standards and urine are presented. 1, MMA; 2, DMA; 3, TMA; 4, propylamine (added intentionally).

It was found that humans excrete methylamines in their urine after they ingest choline-containing compounds (fig. 2). When they consume a normal diet their urinary output of MMA, DMA and TMA is approximately 1 mmol of each amine per day. After consumption of 27 mmol of choline chloride, humans excreted almost 2 mmol/day of MMA and DMA, and more than 17 mmol/day of TMA ($P < .01$ all values different from control). Ingestion of 27 mmol of choline stearate caused humans to excrete DMA (1.2 mmol/day; $P < .05$ different from control) and TMA (9.3 mmol/day; $P < .01$ different from controls). When humans ate 27 mmol of lecithin they excreted more DMA (1.7 mmol/ml; $P < .01$) and TMA (3.8 mmol/day; $P < .01$) than did controls. Methylamine excretion was not increased as much when the humans ingested "cleaned" lecithin (MMA 0.8 mmol/day, DMA 1.0 mmol/day, TMA 2.2 mmol/day).

The ingestion of choline-containing compounds also caused rats to excrete methylamines in their urine (fig. 3). Rats consuming a choline-free diet excreted 0.015 to 0.018 mmol/day of MMA and DMA and 0.004 to 0.009 mmol/day of TMA in their urine. When rats were fed 2.0 mmol of choline chloride they excreted more TMA in their urine (0.02 mmol/day; $P < .01$ different from control). Administration of 2.0 mmol of lecithin also increased TMA excretion (to 0.045 mmol/day). The same amount of cleaned lecithin, when administered to rats, did not result in any increase in methylamine excretion (fig. 4).

It was found that all of the lecithins that we tested were contaminated with significant amounts of TMA. Some preparations also contained DMA (see table 1).

Discussion

Humans and rats excrete methylamines in their urine after they eat choline or lecithin. Asatoor and Simenhoff (1965) noted that DMA concentration was increased in the urine of rats and humans fed very large doses of choline (their doses were more than 5-fold greater than ours). They also found that dimethylglycine, betaine, dimethylethanolamine and carnitine did not have this effect. By using a radioisotopic method, they determined that MMA could be methylated and TMA could be demethylated, forming DMA. The colorimetric assay for DMA used by these authors was probably not as specific as our gas chromatograph method, and it was not able to measure TMA, the major metabolite formed. In our hands this method consistently gave much higher values for DMA than we obtained by gas chromatography. We found that other amines found in urine (such as pyrrolidine and piperidine) contributed to the DMA values obtained using the colorimetric technique.

When choline is administered to humans, exposure to methylamines is certainly increased. This is troublesome because these methylamines could be substrates for the formation of nitrosamines. Nitrosamines have marked carcinogenic activity in a wide variety of animal species (Magee, *et al.*, 1976; Lijinsky

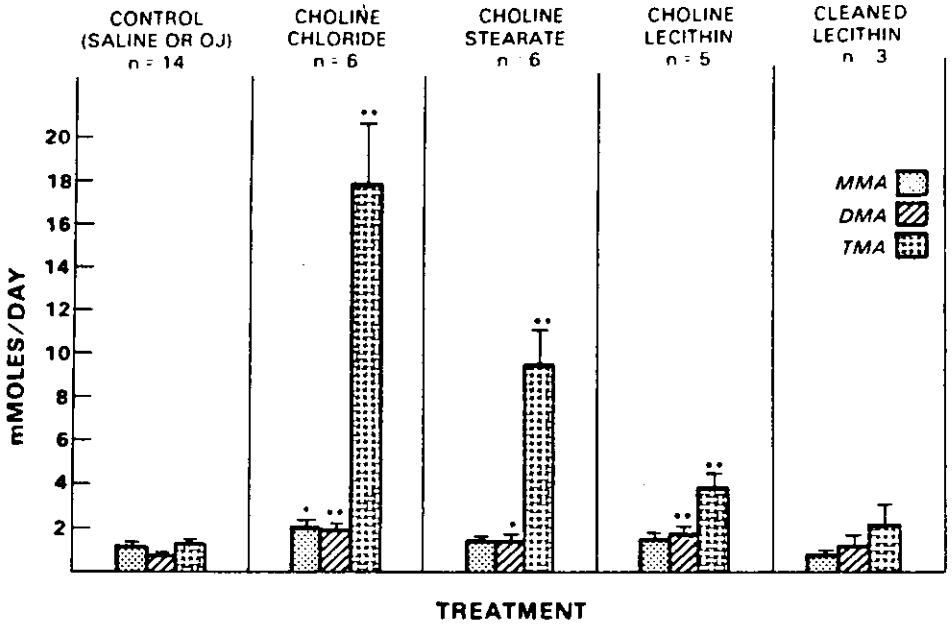

Fig. 2. Effects of choline and lecithin administration on the excretion of methylamines in human urine. Humans consumed either 3.5 g of choline chloride, 10.2 g of choline stearate, 20 g of soy lecithin, 20 g of cleaned soy lecithin or placebo. Urine was collected for a 24-hr period, and an aliquot was analyzed for MMA, DMA and TMA content using a gas chromatographic method. Data are expressed as mean ± S.E., and statistical analysis was performed using one-way analysis of variance. * $P < .05$; ** $P < .01$.

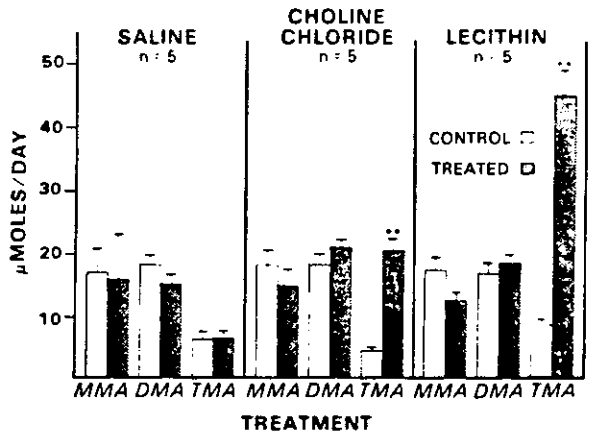

Fig. 3. Effects of the administration of single doses of choline and lecithin on the excretion of methylamines in rat urine. Rats were fed a choline-free diet for 48 hr. During hour 49, rats were fed choline chloride or lecithin (both delivering 200 mg of free choline base per kg b.wt.), or 2 ml of saline. Urine was collected for the 24-hr period before the dose (control period), and for the 24-hr period after the dose was administered (treatment period). An aliquot of each urine sample was analyzed for MMA, DMA and TMA content using a gas chromatographic method. Data are expressed as mean ± S.E., and statistical analysis was performed using one-way analysis of variance. ** $P < .01$. Five rats were used in each group.

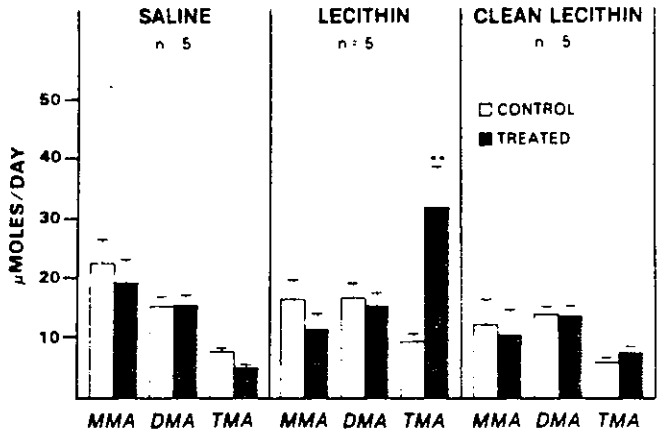

Fig. 4. Effect of cleaning lecithin upon excretion of methylamines in rat urine. Rats were fed a choline-free diet for 48 hr. During hour 49, rats were fed soy lecithin, cleaned soy lecithin (methylamines were removed as described under "Materials and Methods") or 2 ml of saline. Lecithin doses delivered 200 mg of free choline base per kg b.wt. Urine was collected for the 24-hr period before the dose (control period), and for the 24-hr period after the dose was administered (treatment period). An aliquot of each urine sample was analyzed for MMA, DMA and TMA content using a gas chromatographic method. Data are expressed as mean ± S.E., and statistical analysis was performed using one-way analysis of variance. ** $P < .01$. Five rats were used in each group.

and Epstein, 1970). The noxious smell associated with TMA excretion has been a disturbing side effect of choline administration and has led researchers to look for other ways to treat patients. One such alternative has been lecithin. Humans treated with lecithin do not smell like rotten fish. We assume that this is because the amount of TMA excreted from the bodies of these patients is too small to be detected by our noses. However, we cannot eliminate the possibility that the methylamines present in humans ingesting lecithins are substrates for the formation of nitrosamines. When commercial lecithins are reacted with sodium nitrite at pH 5.6, dimethylnitrosamine is formed (Pensabene, *et al.*, 1975). We postulate that the methylamine contaminants in lecithin react with the nitrite. Lecithin is soluble in organic solvents, whereas the methylamines are soluble in water. Therefore, removal of these contaminants should be easy. It is possible that the methylamines are formed after the lecithin is manufactured, perhaps because of bacterial action during storage. We used all the lecithins in the form in which they are made available to humans and we stored them at 4°C. We suggest that lecithin manufacturers should determine which method of storage is optimal for their preparation.

We do not know whether the consumption of choline and lecithin as part of normal foodstuffs results in increased excretion of methylamines. Foods, such as fish and vegetables, often contain TMA (Neurath, *et al.*, 1977; Singer and Lijinsky, 1976). We did note that humans and rats which consume normal diets also excrete some methylamines. It is possible that the choline

and methylamines present in common foods are the source of these urinary amines.

Acknowledgments

We wish to thank Ms. Patricia Michel for her expert technical assistance. Dr. Richard Wurtman and Dr. Steven Tannenbaum of the Massachusetts Institute of Technology gave us generous help and advice.

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