Pharmacology Biochemistry & Behavior, Vol. 30, pp. 249–253. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Recent Studies of Nicotine Metabolism in Humans[1]

PEYTON JACOB, III,[2] NEAL L. BENOWITZ AND ALEXANDER T. SHULGIN

Division of Clinical Pharmacology of the Department of Medicine and Langley Porter Psychiatric Institute, University of California, San Francisco San Francisco General Hospital Medical Center, San Francisco, CA 94110

JACOB, P., III, N. L. BENOWITZ AND A. T. SHULGIN. *Recent studies of nicotine metabolism in humans.* PHARMACOL BIOCHEM BEHAV **30**(1) 249–253, 1988.—Recent studies of quantitative and qualitative aspects of nicotine metabolism, and the use of metabolic data for estimating nicotine intake in humans are discussed. Previously reported methodology for determining nicotine bioavailability has been improved by using stable isotope-labeled nicotine administered intravenously. Combined gas chromatography-mass spectrometry with selected ion monitoring has been used to simultaneously determine concentrations of isotopically-labeled nicotine and tobacco-derived nicotine in blood. Nicotine intake from cigarette smoking was estimated from clearance of labeled nicotine and blood concentrations of tobacco-derived nicotine. Progress in elucidating the metabolic profile of nicotine in humans, and the use of nicotine metabolites as markers of tobacco smoke consumption is summarized.

Nicotine Cigarette smoking Deuterium labeled nicotine Trans-3′-hydroxycotinine

COMPLETE characterization of the metabolism and disposition of a drug requires the determination of the rate of metabolism (pharmacokinetics), elucidation of the structures of the various metabolites, quantitative assessment of the various metabolic pathways, and determination of the urinary excretion profile, i.e., the percentage of the dose accounted for by each metabolite and parent drug excreted in the urine.

Despite the widespread use of tobacco, our knowledge of nicotine metabolism is incomplete. In recent years, considerable information on the rate of nicotine metabolism has been obtained [2–6, 11, 13]. A number of metabolites have been structurally characterized, largely due to the pioneering work of McKennis and co-workers in the 1960's [9,15] Some information on the quantitative importance of various metabolic pathways has been obtained, but at the present time we can account for only about 25% of ingested nicotine as urinary metabolites [3]. Nevertheless, the nicotine metabolic data which is available has proven useful to researchers in assessing the health consequences of tobacco use. A primary application of metabolic data has been the estimation of nicotine intake [2,4,5]. Nicotine differs from therapeutic drugs in that with tobacco use the dose of nicotine is not known. Consequently, there has been considerable interest in methods for estimating intake of nicotine by consumers of various tobacco products. In this paper we will discuss recent studies of nicotine metabolism in humans with emphasis on quantitative aspects of nicotine metabolism, and on the use of metabolic data in estimating intake of nicotine.

GENERAL METHOD

The clinical studies were carried out in subjects hospitalized on the General Clinical Research Center at San Francisco General Hospital Medical Center. Methods for measuring clearance and intake of nicotine have been described previously [4]. Concentrations of nicotine and cotinine in blood and urine were determined by gas chromatography [17]. Simultaneous determination of nicotine-3′,3′-d_2 and unlabeled nicotine in blood was carried out by combined gas chromatography-mass spectrometry (GC-MS) with selected ion monitoring. Details of the synthesis of deuterium-labeled nicotine and the GC-MS assay will be reported elsewhere. Identification of trans-3′-hydroxycotinine in a sample of pooled smokers' urine was made by GC-MS with the mass spectrometer operated in the scan mode, and comparison of the mass spectrum with an authentic standard obtained by synthesis [10].

One subject (male, age 43) smoked two cigarettes (1.0 mg FTC nicotine yield) over the course of 15 min. After finishing the second cigarette, nicotine-3′,3′-d_2 bitartrate was infused intravenously for 30 min at 2.0 μg/kg/min. Blood samples

[1]Supported in part by U.S. Public Health Service Grants DAO2277, CA32389 and DAO1696. Studies were carried out in part in the General Clinical Research Center (RR-00083) with support of the Division of Research Resources, National Institutes of Health.

[2]Requests for reprints should be addressed to: Peyton Jacob, III, Ph.D., San Francisco General Hospital Medical Center, Building 100, Room 235, 1001 Potrero Avenue, San Francisco, CA 94110.

were collected at frequent intervals over the course of 6 hr. Total clearance (CL_T) of nicotine-3',3'-d_2 was computed using model-independent methods [1,6]. The area under the blood concentration-time curve (AUC) for nicotine and nicotine-3',3'-d_2 was computed using the trapezoidal rule. Intake of nicotine from cigarettes (D) was computed as $D=(AUC)\times(CL_T)$, using AUC of tobacco-derived nicotine and CL_T calculated from nicotine-3',3'-d_2 infusion data.

Rate of Nicotine Metabolism

There is good evidence that nicotine plays a role in tobacco dependence. Consequently, it is important to understand the quantity and time course of nicotine in the body. Following smoking or an intravenous infusion, nicotine distributes rapidly into tissues, after which it is eliminated, with a terminal half-life of about 2 hr [6]. Total clearance averages about 1300 ml/min and is highly variable between individuals. The relatively long terminal half-life in the presence of rapid clearance can be explained by the large volume of distribution, which averaged 183 liters.

The amount of a drug in the body is a function of both intake (dose) and rate of elimination. Nicotine differs from therapeutic drugs in that the dose is not known, and varies widely depending upon the habits of the individual tobacco user. In addition, the rate of nicotine elimination is highly variable, with clearance rates varying as much as 4-fold among cigarette smokers [6]. Consequently, a fast metabolizer may consume up to four times the dose of nicotine as a slow metabolizer to produce equivalent blood concentrations.

Use of Pharmacokinetic Data in Determining Nicotine Intake

Because of the health consequences of tobacco smoking and the role that nicotine plays in tobacco dependence, there has been considerable interest in methods for estimating nicotine intake. Our approach for estimating nicotine intake has been to determine nicotine clearance following intravenous infusion of nicotine during a period of tobacco abstinence [4]. When the subject resumes smoking, we measure nicotine blood concentrations and urinary nicotine excretion in order to determine the area under the blood concentration-time curve (AUC) and renal clearance. Using clearance determined following intravenous infusion and AUC determined during the smoking period, we can estimate nicotine intake during smoking. This method is analogous to methodology used in drug bioavailability studies. As expected, considerable variability in nicotine intake among cigarette smokers was observed. It is well known that smoking behavior is highly variable, and that smokers can to a remarkable degree regulate their nicotine intake. For 22 subjects studied on a research ward, daily intake of nicotine averaged 37.6 mg with a range of 10.5 to 78.6 mg [4]. Nicotine intake per cigarette averaged 1.04 ± 0.36 mg (range 0.37 to 1.56), and did not correlate with smoking machine yield. Data from a cigarette brand switching study [5] illustrated the applicability of this method and the phenomenon of nicotine regulation. Eleven subjects consumed the same amount of nicotine (mean of 26 mg/day) when smoking either a commercial high-yield cigarette (1.2 mg nicotine by FTC method) or a low-yield cigarette (0.43 mg nicotine by FTC method.).

Use of Stable Isotope Methodology in Determining Nicotine Intake

The method for estimating nicotine intake described above requires that subjects abstain from smoking for at least 8 hours prior to nicotine infusion in order for levels of tobacco-derived nicotine to fall low enough so as not to confound the test infusion of unlabeled nicotine. Since tobacco smoke contains a variety of substances which may influence the rate of nicotine metabolism, e.g., carbon monoxide, hydrogen cyanide, polycyclic aromatic hydrocarbons [16], it is possible that nicotine clearance during tobacco abstinence is different than clearance during smoking. Consequently, it is most desirable to study nicotine metabolism quantitatively under actual smoking conditions. To do so requires administration of isotopically-labeled nicotine in order to distinguish the nicotine given by injection from tobacco-derived nicotine.

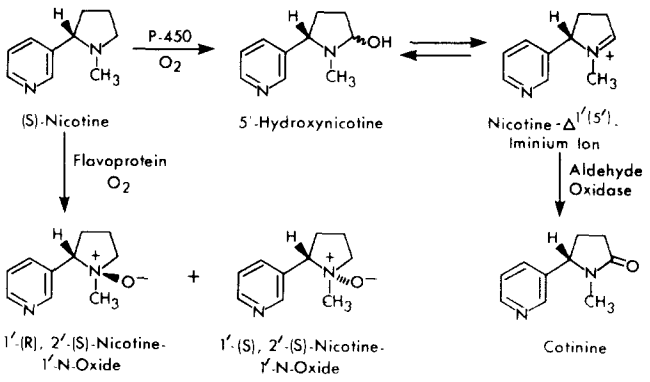

FIG. 1. Oxidative metabolism of nicotine.

One approach would be to use commercially available radiolabeled nicotine, but this would entail some risk to the subjects, as well as requiring tedious separations of nicotine from metabolites in the course of chemical analyses. A preferred approach [12] is to administer stable isotope-labeled nicotine, since this is no more hazardous than administration of unlabeled nicotine, and to use combined gas chromatography-mass spectrometry (GC-MS) to separate nicotine from its metabolites and distinguish the labeled from the unlabeled drug.

Several factors concerning chemistry had to be considered before applying stable isotope methodology to nicotine metabolic studies. Stable isotope-labeled nicotine is not available commercially, and a suitable synthesis had to be devised. Of the various stable isotopes, the hydrogen isotope deuterium is by far the least expensive and generally the easiest to incorporate into organic molecules. If gram quantities are required for pharmacologic studies, the use of other isotopes (e.g., carbon-13, nitrogen-15, oxygen-18) might be prohibitively expensive, especially if multi-step syntheses are required.

Other factors to be considered are the metabolic pathways. If the portion of the molecule bearing the label is lost during metabolism, it will be impossible to distinguish metabolites derived from labeled and unlabeled drugs. In addition, deuterium located at a site of metabolism may result in a slower rate of metabolism due to a kinetic isotope effect [12,18]. Consequently, metabolic pathways must be considered in choosing the site to be labeled. The major routes for nicotine metabolism are oxidation of the 5'-carbon to form cotinine, and N-oxidation to nicotine N'-oxide (Fig. 1). In order to prevent loss of label and minimize chances of an isotope effect, a deuterium label on the 5'-carbon must not be used.

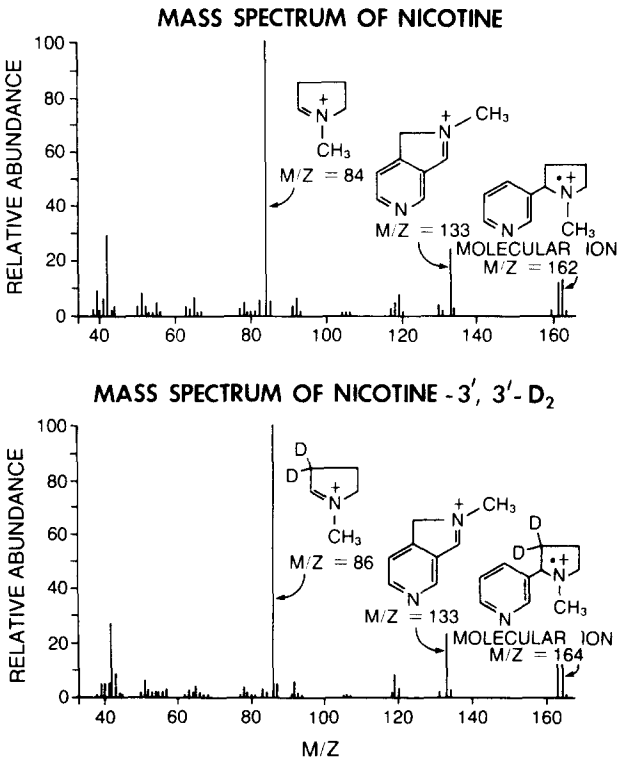

FIG. 2. Mass spectra of nicotine and nicotine-3',3'-d_2.

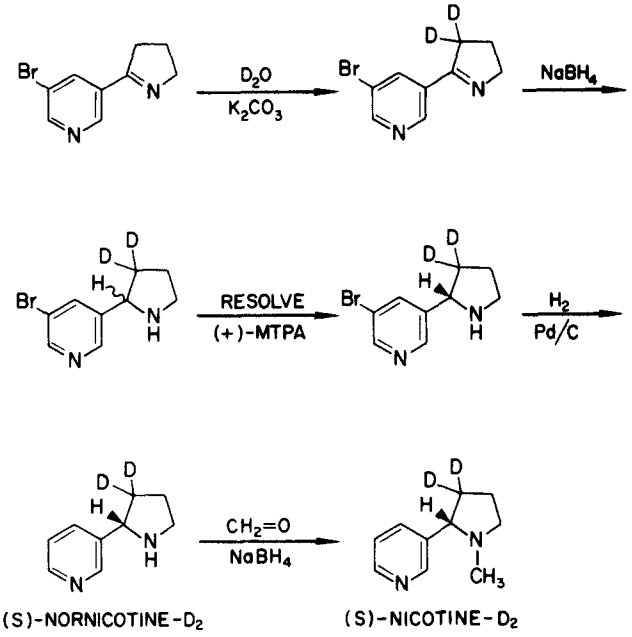

FIG. 3. Synthesis of nicotine-3',3'-d_2.

Another factor that must be considered in choosing the site to be labeled is the mass spectral fragmentation pathway. Quantitative analysis by GC-MS is carried out using selected ion monitoring (SIM), which involves tuning the mass spectrometer to measure certain ions characteristic of the compounds of interest. If it is necessary to monitor fragment ions to achieve maximum sensitivity, as is often the case in electron-impact mass spectrometry, the label must not be lost in their formation.

The mass spectrum of nicotine is presented in Fig. 2. Maximum sensitivity using SIM is achieved by monitoring the most abundant ion (M/Z=84) which results from loss of the pyridine ring. If the isotopic label were located on the pyridine portion of the molecule, both forms of nicotine would give rise to the same mass 84 ion and would be indistinguishable.

For the above reasons, we chose the 3'-position as the site for incorporation of two deuterium atoms. This part of the molecule is not a primary site of metabolism, and is maintained in the most abundant ion formed in electron impact mass spectrometry (Fig. 2).

Finally, a synthetic route providing sufficient quantities of labeled nicotine with correct stereochemistry had to be devised. Nicotine in tobacco is the levorotatory (S)-isomer, although small amounts of the (R)-isomer may be formed pyrolytically during smoking. We have devised a synthesis which provides (S)-nicotine-3',3'-d_2 with enantiomeric purity of >99% (Fig. 3). The synthesis can be carried out on a large scale and may be used to prepare the "unnatural" (R)-isomer as well.

Preliminary data has indicated that nicotine-3',3'-d_2 is a highly satisfactory analog for the proposed metabolic studies. A GC-MS-SIM assay has been developed for simultaneous determination of both labeled and unlabeled nicotine. Figure 4 shows blood concentration-time curves for an intravenous infusion of nicotine-3',3'-d_2 in a subject following smoking of two cigarettes. Total clearance was calculated from the dose and AUC for deuterium-labeled nicotine. The dose of tobacco-derived nicotine was calculated using AUC of nicotine and clearance determined from the infusion. In this subject, 0.96 mg of nicotine was absorbed from smoke, or 0.48 mg per cigarette.

Nicotine Metabolic Pathways

The primary metabolites of nicotine which have been characterized are nicotine N-oxide and cotinine (Fig. 1). About 4% of ingested nicotine is converted to nicotine N-oxide, which is largely if not entirely excreted in the urine without further metabolism. About 70% of nicotine is converted to cotinine. In contrast to nicotine N-oxide, cotinine is extensively metabolized (Figs. 5 and 6), only about 10–15% being excreted unchanged in urine. Several metabolites of cotinine have been reported (Fig. 6) [15], but no quantitative data on these metabolites has been published. We have found that cotinine N-oxide is a minor metabolite, its urinary excretion accounting for only about 3% of nicotine ingested by smokers [20]. At the present time, we can account for only about 25% of ingested nicotine as urinary metabolites and nicotine urinary excretion (Fig. 5) [3].

Nicotine Metabolites as Markers for Tobacco Smoke Exposure

For epidemiologic and behavioral studies, it is desirable to estimate exposure to tobacco smoke. The methodology for determining nicotine intake using clearance data as described above requires hospitalization of subjects to obtain multiple blood samples and is not practical for many studies. Consequently, we [4,5] and others [13,14] have attempted to

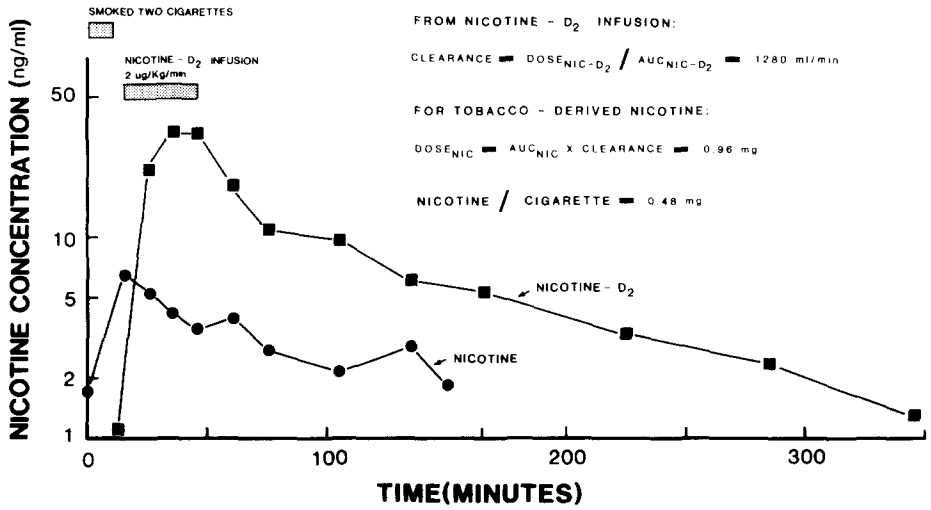

FIG. 4. Blood concentrations of nicotine and nicotine-3′,3′-d_2 after smoking two cigarettes and following intravenous infusion of nicotine-3′,3′-d_2.

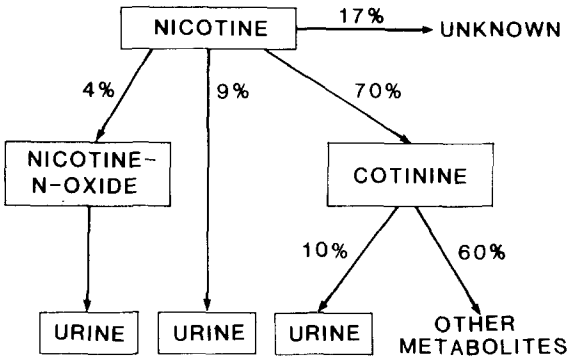

FIG. 5. Quantitative disposition of nicotine in smokers.

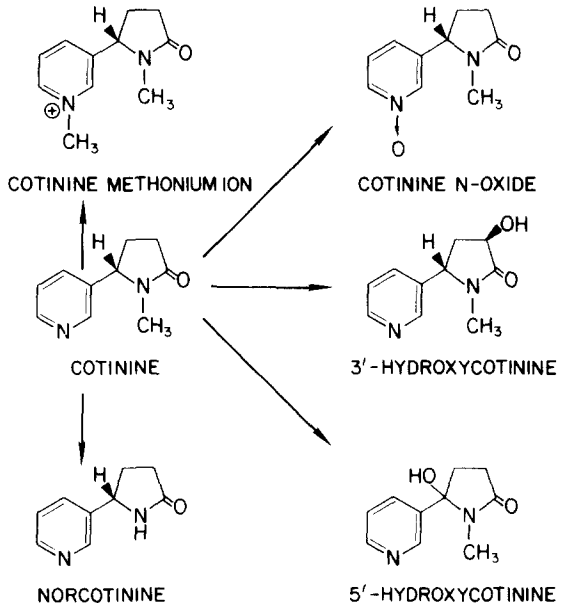

FIG. 6. Cotinine metabolic pathways.

correlate nicotine intake with various markers of tobacco smoke exposure present in biologic fluids of smokers. We have found that, in studies of smokers on a research ward, afternoon nicotine blood concentrations or carboxyhemoglobin correlate fairly well with intake of nicotine [4] (Table 1). Urinary excretion of nicotine correlated poorly with intake, probably a reflection of large interindividual variability and influence of urine pH and flow on renal clearance of nicotine.

Cotinine is generally believed to be the most useful marker of nicotine exposure [14, 19]. In contrast to nicotine, cotinine has a long biologic half-life [8] (ca. 20 hours), and blood concentrations are relatively stable throughout the smoking day. Nicotine concentrations are much more dependent upon sampling time with respect to smoking. Nevertheless, the correlation of cotinine blood concentrations and daily intake of nicotine was only 0.53 (Table 1). This is most likely due to individual differences in the fractional conversion of nicotine to cotinine and rate of cotinine metabolism.

If it were possible to measure the concentrations of nicotine and all of its metabolites excreted in urine, a simple calculation could estimate average daily nicotine intake. Further information about the nature of metabolites and methods for routine determination of concentrations of urinary metabolites would enable us to use urinary metabolite data to quantitate nicotine exposure.

Trans-3′-Hydroxycotinine: A Major Urinary Metabolite of Nicotine

Using GC-MS, we have confirmed that 3′-hydroxycotinine is a nicotine metabolite present in smokers' urine. In addition, we have demonstrated that this metabolite is largely (>98%) the *trans*-isomer (Fig. 6), which was found to be the isomeric form produced by the rhesus monkey [10]. Preliminary experiments indicate that trans-3′-hydroxycotinine is a major metabolite, its urinary concentrations exceeding cotinine concentrations by 2–3-fold. Consequently, a substantial portion of nicotine intake may be accounted for as

TABLE 1

CORRELATION OF VARIOUS MARKERS OF TOBACCO SMOKE WITH DAILY INTAKE OF NICOTINE

Measure	Correlation (r)
Blood nicotine concentration, 1600 hr	0.81*
Carboxyhemoglobin level, 1600 hr	0.69*
Blood cotinine concentration, 1600 hr	0.53†
Urinary cotinine excretion, 24 hr	0.62*
Urinary nicotine excretion, 24 hr	0.39

*$p<0.01$; †$p<0.05$ (from Benowitz and Jacob [4]).

urinary excretion of 3′-hydroxycotinine. This metabolite may be a good marker for tobacco smoke exposure. Work is now in progress to develop a routine assay and correlate nicotine intake with urinary excretion of 3′-hydroxycotinine.

CONCLUSIONS

Estimation of intake of nicotine by tobacco users is useful to researchers studying smoking behavior and nicotine dependency, to epidemiologists assessing the health risks of various tobacco products, and to therapists involved in the treatment of tobacco dependence. There is considerable variability in the way people smoke and, as a result, it is not possible to estimate nicotine intake reliably, based upon the amount of tobacco consumed. Nicotine blood concentrations *per se* are not a reliable reflection of nicotine intake since there is wide interindividual variation in the rate of nicotine metabolism.

We have described the use of nicotine metabolic data in estimation of nicotine intake. The determination of nicotine bioavailability using stable isotope methodology is advantageous since nicotine clearance is determined under actual smoking conditions. However, this requires hospitalization of subjects on a research ward, which would not be possible for some studies. Consequently, there is a need for simpler methods for estimating nicotine intake, particularly noninvasive urine or saliva measures. Studies are in progress to determine quantitatively nicotine metabolism in humans, to develop analytical methodology for metabolites, and to validate new metabolic markers of tobacco smoke consumption.

ACKNOWLEDGEMENTS

The authors are grateful to Chin Savanapridi, Lisa Yu, Margaret Wilson, Lourdes Abayan, Kaye Welch and Gunnard Modin for their assistance in this research.

REFERENCES

1. Benet, L. Z. and R. L. Galeazzi. Noncompartmental determination of the steady state volume of distribution. *J Pharm Sci* **68:** 1071–1074, 1979.
2. Benowitz, N. L. The use of biologic fluid samples in assessing tobacco smoking consumption. In: *Measurement in the Analysis and Treatment of Smoking Behavior*, NIDA Research Monograph 48, edited by J. Grabowski and C. S. Bell. Washington DC: U.S. Government Printing Office, 1984, pp. 6–26.
3. Benowitz, N. L. and P. Jacob, III. Metabolism, pharmacokinetics and pharmacodynamics of nicotine in man. In: *Tobacco Smoking and Nicotine: A Neurobiological Approach*, edited by W. R. Martin, G. R. Van Loon, E. T. Iwamoto and L. Davis. New York: Plenum Press, 1987, pp. 357–373.
4. Benowitz, N. L. and P. Jacob, III. Daily intake of nicotine during cigarette smoking. *Clin Pharmacol Ther* **35:** 499–504, 1984.
5. Benowitz, N. L. and P. Jacob, III. Nicotine and carbon monoxide intake from high- and low-yield cigarettes. *Clin Pharmacol Ther* **36:** 265–270, 1984.
6. Benowitz, N. L., P. Jacob, III, R. T. Jones and J. Rosenberg. Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. *J Pharmacol Exp Ther* **221:** 368–372, 1982.
7. Benowitz, N. L., P. Jacob, III, L. Yu, R. Talcott, S. Hall and R. T. Jones. Reduced tar, nicotine and carbon monoxide exposure while smoking ultralow- but not low-yield cigarettes. *JAMA* **256:** 241–246, 1986.
8. Benowitz, N. L., F. Kuyt, P. Jacob, III, R. T. Jones and A.-L. Osman. Cotinine disposition and effects. *Clin Pharmacol Ther* **34:** 604–611, 1983.
9. Bowman, E. R. and E. R. McKennis. Studies on the metabolism of (−)-cotinine in the human. *J Pharmacol Exp Ther* **135:** 306–311, 1962.
10. Dagne, E. and N. Castagnoli, Jr. Structure of hydroxycotinine, a nicotine metabolite. *J Med Chem* **15:** 356–360, 1972.
11. Darby, T. D., J. E. McNamee and J. M. van Rossum. Cigarette smoking pharmacokinetics and its relationship to smoking behavior. *Clin Pharmacokinet* **9:** 435–449, 1984.
12. Eichelbaum, M., G. E. von Unruh and A. Somogyi. Application of stable labeled drugs in clinical pharmacokinetic investigations. *Clin Pharmacokinet* **7:** 490–507, 1982.
13. Feyerabend, C., R. M. J. Ings and M. A. H. Russell. Nicotine pharmacokinetics and its application to intake from smoking. *Br J Clin Pharmacol* **19:** 239–247, 1985.
14. Galeazzi, R. L., P. Daenens and M. Gugger. Steady-state concentrations of cotinine as a measure of nicotine intake by smokers. *Eur J Clin Pharmacol* **28:** 301–304, 1985.
15. Gorrod, J. W. and P. Jenner. The metabolism of tobacco alkaloids. In: *Essays in Toxicology, Vol 6*, edited by J. Hayes. New York: Academic Press, 1975, pp. 35–78.
16. Hoffmann, D. and E. L. Wynder. *Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis*. New York: Academic Press, 1967.
17. Jacob, P., III, M. Wilson and N. L. Benowitz. Improved gas chromatographic method for the determination of nicotine and cotinine in biologic fluids. *J Chromatogr* **222:** 61–70, 1981.
18. Pohl, L. R. and J. R. Gillette. Determination of toxic pathways of metabolism by deuterium substitution. *Drug Metab Rev* **15:** 1335–1351, 1984–85.
19. Sepkovic, D. W. and N. J. Haley. Biomedical applications of cotinine quantitation in smoking related research. *Am J Public Health* **75:** 663–664, 1985.
20. Shulgin, A. T., P. Jacob, III, N. L. Benowitz and D. Lau. Identification and quantitative analysis of cotinine-N-oxide in human urine. *J Chromatogr* **423:** 365–372, 1987.