

Demethylation in the Metabolism of (–)-Nicotine*

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A number of early studies (4–6) on the metabolism of randomly labeled nicotine- C^{14} have indicated a high degree of urinary excretion of nicotine and its metabolites in the rat, mouse, and dog. In investigations with the latter two species, it was noted that respiratory carbon dioxide contained no radioactivity after administration of the labeled compound. Hucker (7) likewise sought evidence for the metabolism of nicotine to single-carbon components and reported that a fortified liver preparation, although active in the metabolism of nicotine, produced no formaldehyde.

In recent studies from this laboratory, in which a variety of metabolites of (–)-nicotine have been identified (8–10), it was noted (9) that (–)-nicotine was metabolized by dogs to (–)-demethylcotinine by way of the intermediate (–)-cotinine. This latter work, which at once implicated single-carbon metabolites in the catabolism of (–)-nicotine, has led to an investigation of the metabolism of (–)-nicotine with high specific activity of C^{14} in the methyl group.

(–)-Nicotine-methyl- C^{14} was conveniently synthesized by the reaction (11) of (–)-nornicotine with formaldehyde- C^{14} in formic acid. Studies with (–)-nicotine-methyl- C^{14} in the rat clearly show the oxidation of the methyl group of nicotine to carbon dioxide and lead to a consideration of possible routes in this metabolism. Parallel studies on the metabolism of (–)-nicotine-methyl- C^{14} in the dog serve to indicate that the methyl group of (–)-cotinine and that of a subsequent metabolite γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide are derived from the methyl group of nicotine.

EXPERIMENTAL PROCEDURE

Chromatographic Procedures—All paper chromatograms were prepared on Whatman No. 1 paper and developed by the descending method at ambient temperature. For simplicity, the solvent system composed of ammonia-ethanol-butanol (9) is called the “ammonia system.” The composition of the “formic acid system” has been previously described (9). “Koenig-posi-

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tive” areas were disclosed by treating the dried chromatograms with an alcoholic solution of *p*-aminobenzoic acid (2% by weight) and subsequent exposure of the redried chromatograms to gaseous cyanogen bromide.

Before paper chromatography, urine samples (50 to 300 ml) were placed upon a column of Dowex 50 (H^+) containing approximately 1 ml of wet resin for each 2 ml of urine. After a water wash, the resin column was eluted with 2 M ammonia water, the ammoniacal eluates were concentrated at the water pump and then placed upon paper for chromatography.

(–)-Nicotine-methyl- C^{14} —To 30.1 mg of (–)-nornicotine ($[\alpha]_D^{25} -87.86^\circ$) in a 15-ml tube were added 6 mg of formaldehyde- C^{14} (specific activity 5 mc per mmole, New England Nuclear Corporation), 2 ml of water, and 3 drops of 90% formic acid. The stoppered tube was heated in the boiling water bath for 2 hours. The cooled solution was transferred to a 500-ml Kjeldahl flask with 10 ml of water. Two milliliters of 3.6 M acetic acid and 10 ml of sodium nitrite solution (12) were then added. The mixture was kept at room temperature for 20 minutes and then made alkaline to phenolphthalein by addition of 30% (weight by weight) sodium hydroxide and finally neutralized to phenolphthalein by dropwise addition of 3.6 M acetic acid. After addition of pH 10 sodium carbonate-borax buffer (12), the nicotine was steam-distilled into a 250-ml Erlenmeyer flask containing 1 ml of 5 N hydrochloric acid. The distillate was transferred into a 500-ml round-bottomed flask and then concentrated to a small volume at the water pump. Water (50 ml) was then added and subsequently removed in a vacuum. The process was repeated to insure removal of any unchanged formaldehyde. The residual nicotine was chromatographically pure upon paper chromatography in the “ammonia” and “formic acid” systems as shown by autoradiograms which had a single radioactive zone corresponding in R_F value to authentic (–)-nicotine. The total yield of purified (–)-nicotine assayed in aqueous solution by the method of Willits *et al.* (13) was 21.5 mg. Aqueous solutions of the product were treated with chromotropic acid (14) and compared in the colorimeter against standards of pure nicotine to which formaldehyde had been added. The colorimetric values indicated that contamination by formaldehyde, if any, was less than 0.1%. The foregoing procedure (15), which is based on that previously used for the preparation of (–)-, (+)-, and racemic nicotine (16, 17), proves to be more convenient than methylation of nornicotine with methyl iodide- C^{14} (18).

Metabolism of (–)-Nicotine-methyl- C^{14} in Rat—Male albino rats (Wistar strain from Albino Farms, Red Bank, New Jersey), weighing approximately 250 g, were housed in glass metabolism cages and allowed water but no food. (–)-Nicotine-methyl- C^{14} was administered intraperitoneally (0.2 mg/100 g) in

aqueous solution (0.12 mg per ml). Respiratory carbon dioxide was swept by air from the cages into 10% sodium hydroxide solution. Radioactive carbon dioxide was counted as barium carbonate. Urine samples were dried on talc at 90°. Feces were dried at 90°. All samples were counted at infinite thickness. Radioactivity of urea was determined by counting carbon dioxide (as barium carbonate) evolved by the action of urease (19) on samples of urine. Before the treatment with urease, the samples were degassed under acidic conditions. The carbon dioxide from these samples contained trace quantities of radioactivity.

Metabolism of (-)-Cotinine in Rat—Six male albino rats (360 to 500 g) were fasted for 12 hours. The animals were then anesthetized with ether and the anal sphincters were closed with wound clips to prevent fecal contamination of the urine. After recovery from the anesthetic, each animal was given (-)-cotinine (9) (1 g per kg) in an aqueous solution (500 mg per ml) by stomach tube. A total of 2.75 g of (-)-cotinine was thus administered. The animals were then placed in metabolism cages and allowed water but no food during the subsequent 48-hour period. The urine was collected under toluene during this period.

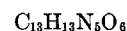
The pooled urine was filtered through Celite and then adjusted to a volume of 500 ml and approximately pH 9 by addition of ammonia water. The solution was exhaustively extracted with chloroform in a rotating extractor. When chromatographed, the chloroform solution showed two Koenig-positive zones, R_F 0.25 and R_F 0.42 ("formic acid" system), and was concentrated to a brown gum. A neutral aqueous solution of this residue was placed upon a column (9 × 1.5 cm) of Dowex 21K (OH⁻). The effluent and water wash were combined (Fraction A) for further processing.

*Isolation and Identification of γ -(3-Pyridyl)- β -oxo-*N*-methylbutyramide from Rat Urine*—The foregoing column was eluted with 1 M acetic acid. Paper chromatography of the acidic solution in the "formic acid" system revealed a single Koenig-positive spot, R_F 0.42, corresponding in value to authentic γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide (10). The solution was concentrated under diminished pressure to 220 mg of reddish brown oil which was dissolved in a small amount of benzene. The solution was placed upon a column (2 × 7.5 cm) of Florisil (Floridin Company, Tallahassee). The column was eluted with 100 ml of acetone-benzene (1:1 by volume) and finally 100% acetone, until no more Koenig-positive material was removed. The combined Koenig-positive eluates were evaporated to a reddish oil. The latter was dissolved in hot acetone. The cooled solution deposited 103 mg of colorless crystals of γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide. The metabolite was recrystallized from benzene to constant melting point, 114–116°. Upon admixture with an authentic sample, m.p. 114–116°, no melting point depression was obtained. The metabolite cochromatographed in the "formic acid" and "ammonia" systems with authentic material.

Identification of Hydroxycotinine and Demethylcotinine as Metabolites of (-)-Cotinine in Rat—Fraction A (see above), when chromatographed in the "formic acid" system, displayed Koenig-positive zones corresponding to (-)-cotinine, R_F 0.42, and demethylcotinine-hydroxycotinine, R_F 0.25. The fraction was concentrated under diminished pressure to a brown oil (1.13 g). To the oil were added 5 ml of acetic anhydride and 3 ml of pyridine. The solution was allowed to stand overnight at room

temperature. The solution was treated with 10 ml of methanol and then concentrated under diminished pressure. The residual brown oil was dissolved in benzene and placed upon a column (2 × 15 cm) of Florisil. The column was eluted with benzene containing increasing amounts of acetone and finally pure acetone. With the "formic acid" system, paper chromatograms of the eluates revealed Koenig-positive components corresponding in R_F value to authentic (-)-cotinine (appearing in the 0 to 85% acetone eluate), acetylcotinine (0 to 30% acetone), and (-)-demethylcotinine (65 to 100% acetone). Occasionally at R_F 0.55 (40 to 70% acetone eluates) the Koenig reaction was positive, yielding an evanescent purplish hue.

Isolation of Demethylcotinine from Rat Urine—Fractions from the foregoing column (85 to 100% acetone and containing a single Koenig-positive zone corresponding to demethylcotinine) were combined and evaporated to a brown oil (48 mg). The oil was dissolved in absolute ethanol and then treated with 52 mg of 5-nitrobarbituric acid in 0.5 ml of ethanol-water (3:1 by volume). The precipitate of (-)-demethylcotinine 5-nitrobarbiturate was recrystallized from ethanol-water, micro m.p. 220–222° with decomposition (34.1 mg). The melting point was not depressed by admixture with an authentic sample prepared by reacting molar quantities of (-)-demethylcotinine (9) and 5-nitrobarbituric acid in ethanol-water, micro m.p. 222–224°, dec. The sample for analysis was dried over KOH at 100° and 1 mm Hg, capillary m.p. 218–220°, dec.



Calculated: C 46.65, H 3.90, N 20.89

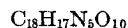
Found: C 46.57, H 4.09, N 20.44

Isolation of Hydroxycotinine and Cotinine from Rat Urine—The fractions from the foregoing Florisil column, which contained Koenig-positive material corresponding in R_F value to (-)-cotinine and acetylcotinine, were combined and concentrated under diminished pressure to 910 mg of light yellow oil. This was dissolved in water and placed upon a column (1 × 6 cm) of Dowex 50 (H⁺). After a period of 1 hour (to allow for hydrolysis of acetylcotinine), the column was eluted with 1 M ammonia water until no more Koenig-positive material was removed. The ammoniacal solution was evaporated under diminished pressure to a light yellow oil (670 mg). A solution of the oil in benzene was placed on a column (2 × 13.5 cm) of Florisil. The column was eluted with benzene, acetone-benzene, and, finally, pure acetone. When chromatographed the 0 to 90% acetone fraction showed, in the "formic acid" system, a single Koenig-positive component corresponding to (-)-cotinine, R_F 0.42, Fraction B. The 90 to 100% acetone fraction, Fraction C, contained a single Koenig-positive zone in the same system, R_F 0.25, corresponding in value to authentic hydroxycotinine (20).

Fraction C was concentrated under diminished pressure to an oil (61 mg). The oil was dissolved in ethanol and treated with 60 mg of picric acid (15% water) as a saturated solution in ethanol. The yellow hydroxycotinine picrate (109 mg) was recrystallized from ethanol, m.p. 132.5–134°. Admixture with an authentic sample of hydroxycotinine picrate (20) caused no depression of melting point.

In a similar experiment on another series of rats, acetylcotinine was separated from demethylcotinine on alumina (9). The acetylcotinine fraction was treated with picric acid to yield a crystalline picrate, m.p. 163–166°, corresponding to that of an

authentic sample. There was no depression of melting point upon admixture.



Calculated: N 15.11

Found: N 14.96

Recovery of Cotinine from Rat Urine—Fraction B (above) yielded, when concentrated under diminished pressure, a light-brown oil (720 mg). The oil was dissolved in ethanol and treated with 300 mg of picric acid (15% water) as a saturated solution in ethanol. The crystalline cotinine picrate (1.01 g) which precipitated was recrystallized from ethanol, m.p. 102°. An authentic sample (21) upon admixture did not depress the melting point.

Administration of (-)-Nicotine-methyl- C^{14} to Dog—A male mongrel dog (9.0 kg), under pentobarbital anesthesia, was given 58.5 mg (6.5 mg per kg) of (-)-nicotine-methyl- C^{14} in 480 ml of 0.9% NaCl via the left femoral vein over a period of 9 hours. Urine was collected by means of an indwelling bladder catheter during administration of the nicotine and a subsequent 15-hour period. When dried on talc at infinite thickness, an aliquot of the pooled urine contained the equivalent of 4.02×10^6 c.p.m. (62.5% of the administered radioactivity).

Fractionation of Radioactivity in Dog Urine—An aliquot of the foregoing urine (50 ml) was adjusted to pH 7 with dilute hydrochloric acid and then concentrated to dryness under diminished pressure. The residue was heated with 100 ml of absolute ethanol under reflux for 2 hours. The insoluble material, which contained 7.1% of the total radioactivity of the sample, was not investigated further. The alcoholic solution (90% of the radioactivity) was concentrated to dryness. The residue was dissolved in water and placed on a column of Dowex 50 (H^+). The effluent and a water wash (Fraction I) contained approximately 3% of the radioactivity; an eluate obtained with 2 M ammonia water (Fraction II) contained 63%; the next eluate from the column obtained with 5 N hydrochloric acid (Fraction III) contained 27%. Fraction III was subjected to further investigation as described elsewhere (22).

Fraction II was concentrated under diminished pressure to a volume of 25 ml and then adjusted to approximately pH 11 by addition of ammonia water for exhaustive extraction with chloroform. The chloroform solution (Fraction IIA), which contained 34% of the radioactivity of the initial sample of urine, was evaporated to dryness. The residue was dissolved in water and placed upon Dowex 21K (OH^-). The effluent and water wash were combined (Fraction IIB). The column was then eluted with 1 M acetic acid to give Fraction IIC.

Isolation of Radioactive Cotinine from Dog Urine—Fraction IIB (above) showed, upon paper chromatography with the "ammonia system," three radioactive and Koenig-positive zones, R_F 0.39, 0.63, and 0.76. The zone at R_F 0.76 was localized by autoradiography on three large chromatograms ($18\frac{1}{2} \times 22\frac{1}{2}$ inches). Cut strips from these zones were extracted with methanol in a Soxhlet extractor. The residue from evaporation of the methanol was dissolved in water and passed onto a column of Dowex 50 (H^+). After a water wash the column was eluted with 2 M ammonia water. The ammoniacal solution upon evaporation yielded a brown gum. To this were added 74.5 mg of (-)-cotinine hydriodide (20) and 1 drop of dilute hydriodic acid. The mixture was dried under diminished pressure and

then dissolved in absolute ethanol. The first crop of crystalline (-)-cotinine hydriodide, micro m.p. 194–197°, specific activity at infinite thinness 5.91×10^4 c.p.m. per mg, was recrystallized to a constant specific activity, 5.51×10^4 c.p.m. per mg (15 mg, micro m.p. 195–197°).

The foregoing hydriodide plus the mother liquor from two recrystallizations (equivalent to a total of 45 mg of (-)-cotinine hydriodide, 5.51×10^4 c.p.m. per mg) were combined and placed in water on a column of Dowex 50 (H^+). After a water wash, the column was eluted with 2 M ammonia water. When evaporated, the ammoniacal solution yielded 30.2 mg of colorless cotinine. The latter was dissolved in a slight excess of dilute hydrobromic acid. After addition of 34.8 mg of (-)-cotinine hydrobromide (20), the mixture was concentrated to dryness. The residue was recrystallized twice from isopropanol to give 32.2 mg, m.p. 187–188°, with constant specific activity at infinite thinness, 4.10×10^4 c.p.m. per mg.

*Isolation of γ -(3-Pyridyl)- β -oxo-*N*-methylbutyramide from Dog Urine*—Fraction IIC (obtained from another aliquot of urine, 75 ml) as described above was concentrated under diminished pressure. The residue (4.1 mg), containing 5% of the total radioactivity of the original aliquot in methanol, was chromatographed on paper with butanol-acetic acid-water (100:22:50 by volume) (23). Autoradiograms revealed the presence of three radioactive zones, R_F 0.16, 0.58, and 0.72. The latter zone of major activity cochromatographed with authentic γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide. The cut zones at R_F 0.72 were extracted with methanol in a Soxhlet extractor. The residue from evaporation of the methanol was dissolved in water. The aqueous solution was placed on a column of Dowex 50 (H^+). After a water wash the column was eluted with 2 M ammonia water. To the residue obtained by evaporation of the solvent were added 100 mg of carrier γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide. The keto amide was recrystallized twice from benzene to give a product weighing 52.4 mg, m.p. 117.5–119°, and constant specific activity at infinite thinness, 1.17×10^4 c.p.m. per mg.

*Methylamine- C^{14} by Hydrolysis of Metabolic γ -(3-Pyridyl)- β -oxo-*N*-methylbutyramide in Vitro*—To 43.0 mg of the foregoing γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide was added new carrier γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide (57 mg). The mixture was heated under reflux in 2 ml of 5 N hydrochloric acid for 5½ hours. The cooled solution was frozen in liquid nitrogen. To the mixture were added 1.1 ml of 10 N sodium hydroxide. With cautious thawing, methylamine and water were then distilled from the mixture into one equivalent of picric acid in a receiver cooled in liquid nitrogen.

After removal of solvent the resulting methylamine picrate was recrystallized twice from isopropanol, 73 mg, m.p. 209°, specific activity at infinite thinness, 3.62×10^8 c.p.m. per mg. Two subsequent recrystallizations produced a constant specific activity of 4.15×10^8 c.p.m. per mg with no change of melting point.

γ -(3-Pyridyl)- β -oxobutyric Acid—The dry residue from the hydrolysis of the keto amide and distillation of the methylamine (above) was triturated with absolute ethanol. The alcoholic solution was filtered and then concentrated to dryness. An aqueous solution of the residue was placed on a column of Dowex 50 (H^+). After a water wash the column was eluted with 2 M ammonia water. The residual γ -(3-pyridyl)- β -oxobutyric acid from evaporation of the ammoniacal solution con-

TABLE I

Metabolism of (-)-nicotine-methyl-C¹⁴ in the male albino rat

Rat No. and weight	Per cent of radioactivity excreted				
	Respiratory CO ₂				Urea (24-hr total)
	0-6 hr	6-12 hr	12-24 hr	Total	
1 (254 g)	7.5	3.2	2.1	12.8	0.26
2 (234 g)	4.0	1.2	1.0	6.2	0.17
3 (260 g)	5.6	1.8	1.0	8.4	0.24

tained no radioactivity and was recrystallized twice from absolute ethanol, m.p. 161-162° (43 mg). The melting point corresponded to that of an authentic sample (10). Admixture of the two caused no depression of the melting point.

Radioactivity of Nicotine and Methylamine Fractions of Dog Urine—An aliquot (5 ml) of urine from the dog which received (-)-nicotine-methyl-C¹⁴ was placed in a 50-ml flask and then frozen in liquid nitrogen. Sodium carbonate (200 mg) was added. The mixture was distilled at room temperature under diminished pressure into a receiver containing 1 ml of 0.5 M hydrochloric acid, cooled by liquid nitrogen. The receiver was slowly thawed with careful mixing of contents and then concentrated to dryness at room temperature in a vacuum. The residual solids (13.4 mg) were dissolved in 2 ml of methanol and then chromatographed (24) with a solvent system: *n*-butanol, concentrated HCl, and water (10:2:3 by volume). The chromatograms were exposed to x-ray film over a 6-day period. No radioactivity was discernable at the zone *R_F* 0.41, corresponding to that of authentic methylamine. The zone corresponding in *R_F* value to authentic nicotine (*R_F* 0.29) was strongly radioactive and contained the equivalent of 3.9% of the administered radioactivity. In an experiment in which control urine was fortified with 12 μg of methylamine hydrochloride-C¹⁴, 95% of the added radioactivity was recovered. Comparative chromatograms indicated that radioactive metabolites with activity of 0.1% of the administered activity were easily located on the autoradiograms.

RESULTS AND DISCUSSION

Determinations on the C¹⁴ concentration in expired air and urea of rats after administration of (-)-nicotine-methyl-C¹⁴ (Table I) show definitely that the methyl group of (-)-nicotine is a precursor of carbon dioxide in this species. It seems sufficiently clear that for the dog the general equations established for the metabolism of nicotine, schematically shown in Fig. 1, give ample opportunity for the formation of carbon dioxide from the methyl group of nicotine and, indeed, other carbons. The fact that previous workers were unable to find C¹⁴ activity in expired air after administration of randomly labeled nicotine-C¹⁴ to the dog can be ascribed presumably to the low activity of their samples.

In the present study it was shown that in the dog (-)-cotinine arising from the metabolism of (-)-nicotine-methyl-C¹⁴ contains radioactivity. Since (-)-cotinine is degraded to (-)-demethylcotinine in the dog, the demethylation of the radioactive cotinine would lead in the present case to C¹⁴ activity in the respiratory carbon dioxide. As previously discussed (10), any degradation of γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide-*N*-methyl-C¹⁴ to the corresponding keto acid would give rise to methylamine-C¹⁴.

Owen and Larson (6), in their studies on the metabolism of randomly labeled (-)-nicotine-C¹⁴, sought evidence for radioactivity in the methylamine fraction from dog urine. Their negative results, but not their interpretation, were confirmed in the present investigation. Although methylamine has been reported as a normal constituent of dog urine (25) and subjected to various limited studies on several occasions (see, for example, references (26) and (27)), there are little or no data on turnover rates of the compound. In unpublished studies from this laboratory conducted in collaboration with Dr. Antonio Horvath, it was observed that the major part of methylamine-C¹⁴ is eliminated as respiratory carbon dioxide soon after administration to the rat. These data add significance to the early experiments in which it was reported that liver preparations would metabolize both nicotine (28) and methylamine (29).

Truhaut and de Clercq (30) noted that (-)-nicotine was metabolized in the rat to a variety of substances. These authors

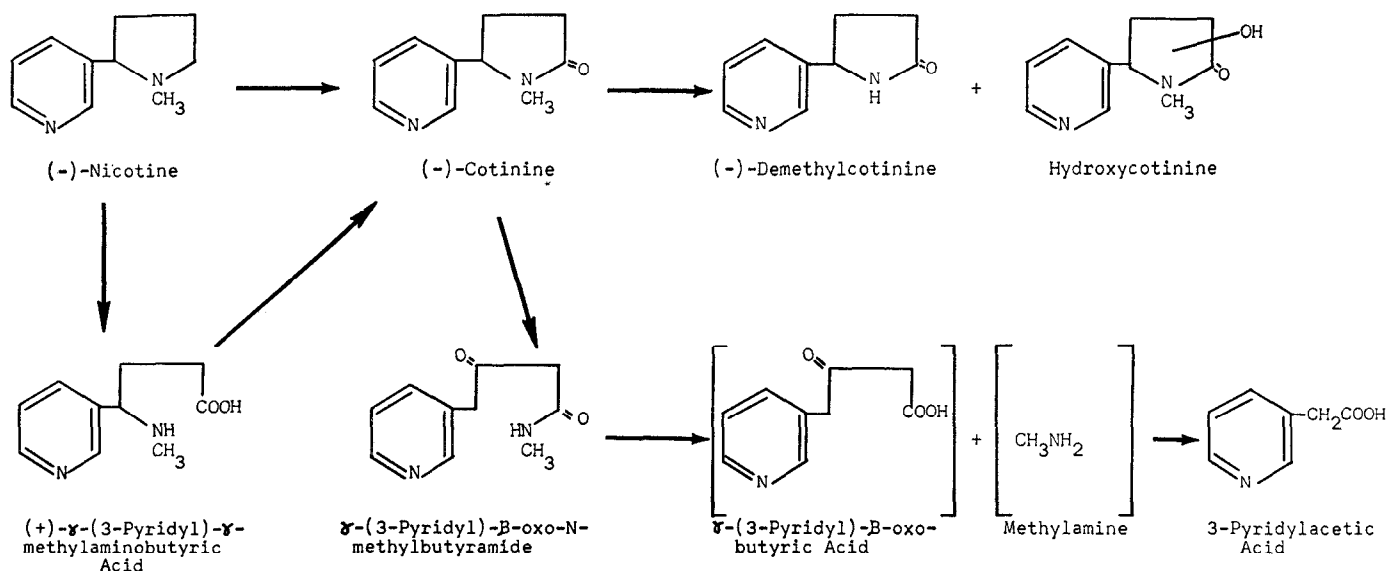


FIG. 1. Nicotine metabolism in the dog.

reported the presence of a small amount of cotinine among other urinary metabolites which were not identified. In the present investigation, the metabolism of (-)-cotinine in the rat was studied at a variety of dose levels. The data obtained serve to suggest that metabolic routes for the degradation of cotinine in the rat resemble those of the dog.

A chloroform extraction of the alkalinized urine obtained from rats after administration of (-)-cotinine contained, upon paper chromatography, Koenig-positive zones corresponding to cotinine- γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide and hydroxycotinine-demethylcotinine.

By chromatography on ion exchange resins and adsorption chromatography, all three of the foregoing metabolites and cotinine were obtained from the urine and chemically identified. It seemed to be especially significant that paper chromatograms of urine from rats given single oral doses of (-)-cotinine (4.0 mg per kg) showed clear signs of demethylcotinine. Human urine (20) obtained under comparable conditions after oral doses of cotinine (3.8 mg per kg and 7.6 mg per kg) showed no evidence of demethylcotinine.

The variety of Koenig-positive components, not extractable with chloroform from alkalinized rat urine, which arises after the administration of cotinine (Table II) also parallels in many respects that obtained after administration of cotinine to both the dog and the human (20). These paper chromatograms, which indicate the presence of at least seven Koenig-positive components and the chloroform-soluble metabolites which have been identified indicate, in view of the reported large number of unidentified urinary metabolites of nicotine in the rat (30), that cotinine serves in this species, as in many others, as an important intermediate in the metabolism of (-)-nicotine.

Of particular interest among the many unidentified components arising from the metabolism of (-)-cotinine is a substance with R_F 0.65 in the "formic acid" system. Material at this zone gave both a positive Koenig reaction and a positive test with 2,4-dinitrophenylhydrazine for carbonyl groups (31). It

was present in both dog and rat urine and cochromatographed with authentic γ -(3-pyridyl)- β -oxobutyric acid (10). Like this keto acid, the metabolic material was extractable from aqueous solution at pH 6 by chloroform. The β -oxo acid or material with these properties has thus far never been chemically identified among the metabolites of nicotine and cotinine. An extended study of the structure and occurrence of this component seems to be warranted.

SUMMARY

The metabolism of (-)-nicotine-methyl- C^{14} has been studied in both the rat and the dog. After administration to the former species, 6 to 10% of the radioactivity of the dose appeared as respiratory carbon dioxide. An examination of the metabolism of (-)-nicotine and the intermediate (-)-cotinine in both species leads to the conclusion that the conversion of cotinine to demethylcotinine is involved in the formation of carbon dioxide- C^{14} . After administration of nicotine-methyl- C^{14} , cotinine and γ -(3-pyridyl)- β -oxo-*N*-methylbutyramide were isolated from the urine of dogs. All of the radioactivity of the latter compound was found in the *N*-methyl group. As a consequence of this and other considerations, methylamine arising from the hydrolysis of the keto amide *in vivo* may be an intermediate in the formation of the carbon dioxide- C^{14} observed in the studies.

REFERENCES

- TURNBULL, L. B., BOWMAN, E. R., AND MCKENNIS, H., JR., *Federation Proc.*, **20**, 172 (1961).
- MCKENNIS, H., JR., WADA, E., BOWMAN, E. R., AND TURNBULL, L. B., *Nature*, **190**, 910 (1961).
- SCHWARTZ, S. L., BOWMAN, E. R., AND MCKENNIS, H., JR., *Virginia J. Sci.*, in press.
- GANZ, A., KELSEY, F. E., AND GEILING, E. M. K., *J. Pharmacol. Exptl. Therap.*, **103**, 209 (1951).
- BENNETT, D. R., TEDESCHI, R. E., AND LARSON, P. S., *Arch. intern. pharmacodynamie*, **98**, 221 (1954).
- OWEN, F. B., JR., AND LARSON, P. S., *Arch. intern. pharmacodynamie*, **115**, 402 (1958).
- HUCKER, H. B., *J. Pharmacol. Exptl. Therap.*, **122**, 33A (1958).
- MCKENNIS, H., JR., TURNBULL, L. B., AND BOWMAN, E. R., *J. Am. Chem. Soc.*, **79**, 6342 (1957).
- MCKENNIS, H., JR., TURNBULL, L. B., BOWMAN, E. R., AND WADA, E., *J. Am. Chem. Soc.*, **81**, 3951 (1959).
- MCKENNIS, H., JR., BOWMAN, E. R., AND TURNBULL, L. B., *J. Am. Chem. Soc.*, **82**, 3974 (1960).
- SPATH, E., HICKS, C. S., AND ZAJIC, E., *Ber. deut. chem. Ges.*, **68**, 1388 (1935).
- MARKWOOD, L. N., *J. Assoc. Offic. Agr. Chemists*, **26**, 283 (1943).
- WILLITS, C. O., SWAIN, M. L., CONNELLY, J. A., AND BRICE, B., *Anal. Chem.*, **22**, 430 (1950).
- MACFAYDEN, D. A., WATKINS, H. D., AND ANDERSON, P. R., *J. Biol. Chem.*, **158**, 107 (1945).
- ESCHWEILER, W., Deutsches Reichs-Patent 80520 (1893).
- HICKS, C. S., AND SINCLAIR, D. A., *Australian J. Exptl. Biol. Med. Sci.*, **25**, 83 (1947).
- SPATH, E., AND ZAJIC, E., *Ber. deut. chem. Ges.*, **68**, 1667 (1935).
- LEETE, E., AND BELL, V. M., *J. Am. Chem. Soc.*, **81**, 4358 (1959).
- VAN SLYKE, D. D., *J. Biol. Chem.*, **73**, 695 (1927).
- BOWMAN, E. R., AND MCKENNIS, H., JR., *J. Pharmacol. Exptl. Therap.*, in press.
- BOWMAN, E. R., TURNBULL, L. B., AND MCKENNIS, H., JR., *J. Pharmacol. Exptl. Therap.*, **127**, 92 (1959).
- TURNBULL, L. B., BOWMAN, E. R., AND MCKENNIS, H., JR., *Federation Proc.*, **19**, 268 (1960).
- STEPKA, W., in A. C. Corcoran (Editor), *Methods of medical research*, The Year Book Publishers, Chicago, 1952, p. 25.

TABLE II

Comparative R_F values of Koenig-positive components* from the metabolism of (-)-cotinine

Animal and oral dose			
Male albino rat			Male mongrel dog
4.7 mg/kg	150 mg/kg	1000 mg/kg	150 mg/kg
R_F	R_F	R_F	R_F
0.03	0.03	0.03	0.05
0.08	0.07		
		0.18	0.22
0.31	0.29	0.29	0.28
0.37†	0.34†	0.37†	0.35†
0.46	0.46	0.46	0.46
0.65‡	0.65‡	0.69‡	0.68‡

* After exhaustive extraction with chloroform at pH 9 to 10, urine samples were placed upon Dowex 50 (H^+). Eluates were obtained with 1 M ammonia water and then, after concentration under diminished pressure, chromatographed in the "formic acid system."

† Cochromatographed with 3-pyridylacetic acid (32).

‡ Cochromatographed with γ -(3-pyridyl)- β -oxobutyric acid.

24. GLOCK, E., AND VAUGHAN, T. H., JR., Tobacco Chemists' Research Conference, Lexington, Kentucky, Oct. 29-30, 1959.
25. ORMSBY, A. A., AND JOHNSON, S., *J. Biol. Chem.*, **187**, 711 (1950).
26. GEORGI, F., HONEGGER, C. G., JORDAN, D., REIDER, H. P., AND ROTTENBERG, M., *Klin. Wochschr.*, **34**, 799 (1956).
27. DAVIS, E. J., AND DE ROPP, R. S., *Nature*, **190**, 636 (1961).
28. WERLE, E., AND MEYER, A., *Biochem. Z.*, **321**, 221 (1950).
29. SCHIEVELBEIN, H., AND WERLE, E., *Arzneimittel-Forsch.*, **7**, 117 (1957).
30. TRUHAUT, R., AND DE CLERCQ, M., *Bull. soc. chim. biol.*, **41**, 1693 (1959).
31. BLAND, D. E., *Nature*, **164**, 1093 (1949).
32. MCKENNIS, H., JR., BOWMAN, E. R., AND TURNBULL, L. B., *Proc. Soc. Exptl. Biol. Med.*, **107**, 145 (1961).

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