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THE FATE AND DISTRIBUTION OF 1-(3-PYRIDYL)ETHANOL METHIODIDE IN RELATION TO THE TOXICITY OF 1-(3-PYRIDYL)ETHANOL AND 3-ACETYLPYRIDINE*

JOHN P. BEDERKA, JR., ESKIL HANSSON, EDWARD R. BOWMAN and HERBERT MCKENNIS, JR.

Department of Pharmacology, Medical College of Virginia, Richmond, Va., U.S.A.
and
Royal Veterinary College, Stockholm, Sweden

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Abstract—(+,−)-N-Methyl-^{14}C-3-(1-hydroxymethyl)pyridinium iodide, prepared from methyl-^{14}C iodide and (+,−)-1-(3-pyridyl)ethanol, was administered intravenously to mice. Sequential whole-body autoradiograms showed a rapid elimination of the radioactivity of (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide from the animals. The compound, in common with the established behavior of many other quaternary ammonium compounds, showed no great tendency to pass the blood–brain barrier. In rats, after intraperitoneal administration, and mice, after intravenous administration, the radioactivity of (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide was eliminated largely (78–90 per cent) by way of the urine within 24 hr. This excreted radioactivity was predominantly in the form of the administered cation.

The signs of acute toxicity from large doses of (+,−)-N-methyl-3-(1-hydroxyethyl)-pyridinium iodide differed from the previously well-established similarity of those arising from 3-acetylpyridine and (+,−)-1-(3-pyridyl)ethanol; and the levorotatory form of 1-(3-pyridyl)ethanol appeared to produce gross toxic effects similar to those from the racemic alcohol.

STUDIES of the effects of 3-acetylpyridine have been attractive to many investigators. The compound—in addition to showing the unusual properties of both niacin and antiniacin activity, according to conditions of the experiments[1, 2]—produces selective brain damage when administered[3, 4] in large doses to rats or mice. Much of this, and other aspects of the biological effects of 3-acetylpyridine, have been reviewed by Herken,[5] Coggeshall and MacLean,[4] and Hicks.[3]

Numerous experiments are recorded[6–12] on the metabolism of 3-acetylpyridine. Recent reports note that 1-(3-pyridyl)ethanol, one of the many metabolites of 3-acetylpyridine, in racemic form produces, after intraperitoneal administration to rats, outward signs of toxicity[10, 13] that are similar to or identical with those observed after administration of 3-acetylpyridine.

Although existing studies do not permit a conclusion about the possible occurrence of optically active forms of 1-(3-pyridyl)ethanol as a result of the metabolism of 3-acetylpyridine in the rat,[10, 11] studies in the dog[12] have resulted in the demonstration

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that the conjugated levorotatory alcohol appears in both the sulfate and glucuronide fractions of urine. Additionally, the racemic form of the N-methyl derivative, (+,−)-N-methyl-3-(1-hydroxyethyl)pyridinium ion, has been isolated from urine in the form of a salt after administration of 3-acetylpyridine to dogs.[8]

In consequence of the foregoing, we were led to investigate some aspects of the toxicology of levorotatory 1-(3-pyridyl)ethanol in comparison with the racemic compound and its N-methyl derivative, and to study the fate of the N-methyl compound, which was administered as (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide to rats and mice.

MATERIALS AND METHODS

Chromatography. Paper chromatograms were developed at room temperature by the descending method on Whatman 1 paper with solvent systems A;[8] E, ethyl acetate: formic acid:water (70:20:10 by vol.); F, tertiary butyl alcohol:methyl ethyl ketone: water:formic acid (44:44:11:0·26 by vol.); G, methanol:water (90:10 by vol.); H, upper phase from saturated aqueous 2-methyl-1-propanol and concentrated aqueous ammonia (100:2 by vol). Thin-layer chromatography was carried out in solvent system J, phenol:water (80:20 by wt.), on glass plates with 250-μ layers of silica gel G (Merck-Darmstadt), applied as an aqueous suspension with two parts of water by weight and activated at 100° for 30 min on the plates. Radioactive zones were located on a Vanguard automatic chromatogram scanner (model 880), in the case of paper chromatograms, and by exposure of thin-layer chromatograms to X-ray film.

Radioactive counting. Radioactivity determinations were made in a scintillation solution containing β-phenylethylamine.[14, 15] Corrections for quenching were made via internal standards or by the channel-ratio procedure.[16–18] Prior to counting, rat feces and other highly colored samples were combusted by the Schoeniger method in a 500-ml Thomas–Ogg combustion flask (Arthur H. Thomas Co., Cat. 6471-P10), modified by insertion of a glass side arm to accommodate a serum bottle stopper at the level of the sample carrier. After combustion, scintillation solution (15 ml) was injected through the stopper and allowed to remain for 1 hr or more before removal of an aliquot (10 ml) for counting. Test determinations on urine samples and standard compounds indicated an agreement of 5 per cent or better between direct-counted and combusted samples, and radioactive paper chromatogram zones which were counted in the scintillator vials gave values within 8 per cent of those obtained by chromatogram scanning. Fecal material from rats was dried at 40° and 10 mm Hg overnight and finely ground prior to the removal of the samples (21–42 mg) for combustion. Mouse feces were counted as a fine suspension in the scintillation solution.

(+,−)-1-(3-*Pyridyl*)*ethanol*

A reduction of 3-acetylpyridine (Aldrich Chemical Co., Inc.) with sodium borohydride afforded (+,−)-1-(3-pyridyl)ethanol. (−)-1-(3-Pyridyl)ethanol was obtained as the salt with L-(+)-tartaric acid, as previously described.[8, 12] The salts employed in the biological investigation had specific rotations, $[\alpha]_{5461}^{25}$ + 2·2 and + 3·0. There were no observed differences in the biological effects of the two samples.

(+,−)-N-*Methyl*-^{14}*C*-3-(1-*hydroxyethyl*)*pyridinium iodide*

The radioactive compound was prepared by a modification of the previously described procedure[8, 12] for N-methylation of 1-(3-pyridyl)ethanol. A sealed ampoule of

(+,−)-1-(3-pyridyl)ethanol (177 mg, 1·44 m-mole) and methyl-^{14}C iodide (108 mg, 0·76 m-mole) in 5·4 ml of 1,2-dimethoxyethane was allowed to stand at room temperature for 1 week. The reaction mixture was treated with a small volume of methanol and decolorizing charcoal (Norit A). After removal of the solvent, the residual oil was seeded with a small crystal of nonisotopic (+,−)-N-methyl-3-(1-hydroxyethyl)-pyridinium iodide. The resultant crystalline mass was washed thoroughly with absolute ether and then dried overnight at 5 mm Hg; yield 147 mg, micro m.p. 80–81° (corr.). Previously recorded capillary melting points are 79°[10] and 85–87°.[8] The product showed a single radioactive zone when chromatographed in solvent systems A, E, F, G, H, and J.

(+,−)-N-*Methyl*-3-(1-*hydroxyethyl*)*pyridinium picrate*

A solution of (+,−)-N-methyl-3-(1-hydroxyethyl)pyridinium iodide (136 mg) in 2 ml water was placed upon a column (3 ml) of Dowex 21K (acetate form). The effluent and excess water wash (neutral to pH test paper) was collected in the presence of 118 mg picric acid (anhydrous). The solution was evaporated to dryness with a stream of air, and the residue was dissolved in 12 ml of warm absolute ethanol. After filtration the solution was concentrated to a volume of approximately 1 ml. The yellow picrate (m.p. 80–81°, 174 mg, or 92 per cent of the calculated amount) formed upon scratching. The product, micro m.p. 80–81°, was dried at 1 mm over KOH and recrystallized from absolute ethanol without change in m.p.: $C_{14}H_{14}N_4O_8$ (366·3). Calculated: C, 45·81; H, 3·85; N, 15·31%. Found: C, 46·02; H, 4·00; N 15·34%.

Autoradiography of animals. The techniques described by Ullberg,[19] with modifications, were employed for the studies. Six male albino mice (22–27 g) received single doses, 4·6 μc (0·34 mg) of (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide, in aqueous solution via the tail vein. At 5 and 20 min, and 1, 4, and 24 hr after injection, the animals were lightly anesthetized with ether and then killed by freezing in dry ice–hexane. The frozen animals were embedded in a solution of carboxymethyl cellulose in water. The sections, eight sagittal (100 μ) and four sagittal (30 μ), were prepared in the cold room at −10° and taken up on Scotch Magic Tape (810 Minnesota Mining & Mfg. Co., St. Paul, Minn.) for drying in the cold room. Apposition autoradiograms were made by pressing the sections against Structurix X-ray film (Gevaert) for 15–127 days. The film was developed in an X-ray developer (Gevaert 230) for 5 min and then fixed. The sections for the figures in this publication were selected for general clarity, on the basis of gross and microscopic observation, and were taken from the vicinity of the midline of the animals. After removal from the films, the sections were stained with hemotoxylin–eosin and mounted in Euparat for detailed histological studies, where required.

Collection of ^{14}C-*activity from rats and mice.* Male albino Wistar-strain rats, previously maintained with Purina rat chow and water *ad libitum*, were injected intraperitoneally with a solution (1 ml) of (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide, and placed in glass metabolism cages with water, but no food. Urine and feces were separated by means of a screen, which permitted some cross-contamination. Respiratory gases were swept in a stream of air into the β-phenylethylamine scintillation solution (400 ml) in a scrubbing tower (6 cm inside diam.), at ice-bath temperature

and equipped with a dispersion tube of pierced polyethylene tubing (5 mm outside diam.).* The scintillation solution was changed every 3 hr.

Respiratory carbon dioxide in the mouse experiments was collected as previously described,[14, 15] and urine was separated from feces by means of a screen which permitted some cross-examination.

Bile excretion. Three male rats (approximately 300 g each) of the Sprague–Dawley strain were anesthetized by intraperitoneal injections of aqueous sodium pentobarbital. The abdomen was opened and a polyethylene tube was inserted into the common bile duct. The animals received (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide (0·07 μg, 0·1 μc) in aqueous solution by a single injection into the femoral vein. The bile was collected during the subsequent 6-hr period.

Urinary excretion of N-*methyl*-^{14}C-3-(1-*hydroxyethyl*)*pyridinium ion.* Aliquots from the diluted 24-hr urines of treated individual mice were added to solutions of weighed carrier (approximately 50–100 mg) (+,−)-N-methyl-3-(1-hydroxyethyl)pyridinium iodide in warm 2-propanol. The combined solution was heated to reflux temperature. After removal of any insoluble material, the solution was reduced in volume. The crystalline N-methyl-3-(1-hydroxyethyl)pyridinium iodide (m.p. 80–81°) was dried under diminished pressure after four or five recrystallizations, counted, and then recrystallized for additional counting.

Aliquots from the diluted 24-hr urines of individual treated rats were chromatographed on large sheets of Whatman 1 paper in solvent G. The radioactive zones, which were located by scanning marginal strips and appeared as single areas in the vicinity of R_f 0·72–0·80, were excised, cut into small pieces, and treated overnight with a methanolic solution of carrier (+,−)-N-methyl-3-(1-hydroxyethyl)pyridinium iodide under reflux. The methanolic solutions were removed and evaporated under a stream of nitrogen. The residual N-methyl-3-(1-hydroxyethyl)pyridium iodide was recrystallized from 2-propanol, with the aid of a seed, and finally from ethanol–ether to obtain the product, which was dried under diminished pressure at 60° for counting, m.p. 80–81°.

Selected samples of the resultant foregoing radioactive (+,−)-N-methyl-3-(1-hydroxyethyl)pyridinium iodide from the experiments on mice and rats were converted to the acetate form on Dowex 21K (acetate) and finally to the picric acid salt, m.p. 80–81°, for confirmation of identity and radioactive content.

General pharmacological experiments. Male Sprague–Dawley rats (150–175 g) and male and female albino mice of the NMRI strain (20–25 g) were used. The compounds were dissolved in physiological saline and injected intraperitoneally. The animals were observed for 7 days. The LD_{50} values in mice (Table 1) were obtained from five or six groups containing ten animals each. The values were calculated according to Litchfield and Wilcoxon.[20]

RESULTS

After the intraperitoneal injection of 3-acetylpyridine (75 mg/kg or more), toxic signs, essentially as described by many previous workers[1, 5, 10] appeared in the rats.

* The tubing proved more convenient than the customary glass frit. It was also found convenient to reduce evaporation and consequent plugging of the discharge holes by passing the effluent gases from the cage into a preliminary, ice-cooled tower containing 82 ml methanol and 138 ml toluene and thence through the phenylethylamine solution.

These signs did not appear in general before 5–6 hr after injection of the compound and took the form of respiratory difficulties, weakness of the hind legs, and a sedation. Some rats, even at the lower dose levels, showed convulsions and died 7–8 hr after the injection. Within 1–2 days all animals died.

The mice employed in our studies showed by and large a lesser outward sensitivity to 3-acetylpyridine. In the limited number of animals studied, with an intraperitoneal dose of 150 mg/kg, no deaths occurred within a 7-day period of observation. The toxic signs in mice were in general similar to, and less pronounced than, those observed in rats. A usual first and persistent sign* in the mice was a closing of the eyes or failure to open the eyelid fully.

The outward toxic signs in both rats and mice after administration of 1-(3-pyridyl)-ethanol—as the racemic base alone, and with 1 M equivalent of L-(+)-tartaric acid, or as the corresponding tartaric acid salt of the levorotatory base—were essentially the same as those that followed administration of 3-acetylpyridine. The acute toxicity in mice (Table 1) and observed signs were ajudged to be independent of the form of

TABLE 1. ACUTE TOXICITY OF 3-ACETYLPYRIDINE, 1-(3-PYRIDYL)ETHANOL, AND N-METHYL-3-(1-HYDROXYETHYL)PYRIDINIUM IODIDE IN MALE MICE

Compound	LD_{50} and (range—95% confidence limits)*	
	(mg/kg)	(m-moles/kg)
3-Acetylpyridine	230 (202–262)	1·90 (1·67–2·16)
(+,−)-1-(3-Pyridyl)ethanol	310 (279–344)	2·52 (2·27–2·80)
(−)-1-(3-Pyridyl)ethanol (+)-acid tartrate	630 (562–706)	2·31 (2·08–2·61)
(+,−)-1-(3-Pyridyl)ethanol (+)-acid tartrate	837 (741–946)	3·06 (2·74–3·50)
(+,−)-N-Methyl-3-(1-hydroxyethyl)pyridinium iodide	700 (619–791)	2·64 (2·33–2·98)

* The LD_{50} was determined in five or six groups with ten animals in each group.

The LD_{50}'s and confidence limits were calculated according to the method of Litchfield and Wilcoxon.[20] A 7-day observation period was employed to ascertain death or survival.

1-(3-pyridyl)ethanol that was employed. It was noted in the mice with 1-(3-pyridyl)-ethanol that the characteristic difficulties in respiration and weakness of the hind legs did not clearly appear until 6–7 hr after injection, approximately 1 hr later than the observed toxic signs following administration of 3-acetylpyridine. When nicotinamide (75 mg/kg) was administered simultaneously by the intraperitoneal route to rats with 3-acetylpyridine (150 mg/kg) or 1-(3-pyridyl)ethanol (150 mg/kg), complete protection was afforded against the otherwise lethal dose of the pyridyl compounds. Nicotinamide (500 mg/kg), injected simultaneously by the intraperitoneal route with 3-acetylpyridine or racemic 1-(3-pyridyl)ethanol (500 mg/kg), provided complete protection against the lethal effects in mice.

The toxicity produced by (+,−)-N-methyl-3-(1-hydroxyethyl)pyridinium iodide followed a course outwardly different in many respects from that provoked by 3-acetylpyridine and its metabolite 1-(3-pyridyl)ethanol. The dominant early sign of toxicity from the methiodide was convulsions; weakness of the hind legs that characterized poisoning by 3-acetylpyridine and 1-(3-pyridyl)ethanol did not appear. In a

* Some of the signs of poisoning are reminiscent of those described for blacktongue in dogs (cf. Ref. 1).

limited number of studies on rats with (+,−)-N-methyl-(1-hydroxyethyl)pyridinium iodide at the higher dose levels necessary to produce death (ca. 1000 mg/kg, i.p.), mortalities were usually encountered within the first 15 min after injection. If death did not occur, the animals recovered and appeared normal in all respects.

The data on mice (Table 1) show no substantial difference in the LD_{50} values for 3-acetylpyridine, (−)-1-(3-pyridyl)ethanol, (+,−)-1-(3-pyridyl)ethanol, and (+,−)-N-methyl-3-(1-hydroxyethyl)pyridinium iodide when the doses of the various compounds are compared on a molar basis.

Tissue distribution of radioactivity from (+,−)-N-*methyl*-^{14}C-3-(1-*hydroxyethyl*)-*pyridinium ion*

General observations. The radioactivity, having left the blood to a large degree, accumulated in the liver, salivary glands, heart, and kidneys within 5 min after the injection. A slight redistribution occurred after 20 min. The concentration in the heart was then lower, although still high in the other above-mentioned organs and the intestines. At 4 hr a concentration of radioactivity was seen in the liver, salivary glands, kidney, and intestinal tract. At 24 hr only the intestinal content showed high activity. The autoradiograms shown in Figs. 1–5 are from sections of mice killed 5 and 20 min, and 1, 4, and 24 hr after injection of the labeled compound. Some of the organs are indicated in the captions. A more detailed, qualitative, descriptive interpretation of some aspects of the autoradiograms, based upon gross and microscopic observations of all sections follows.

The nervous system. No, or only a trace of, radioactivity was observed in the brain, spinal cord, and ischiadic and trigeminal nerves during the 24-hr period of observation.

The circulatory organs. The myocardium, 5 min after the injection, showed a concentration of radioactivity, comparable on the basis of film density to that observed in the liver, salivary glands, intestines, and kidney. A noticeable decline of the concentration in the myocardium was seen 20 min after the injection (Fig. 2).

The digestive system. Radioactivity persisted in the salivary glands during the 24-hr period of observation. From 5 min to 4 hr after the injection the radioactivity was relatively high in the liver. Some radioactivity accumulated during this period in the gall bladder, as seen in a detailed inspection of the films.

Radioactivity was strikingly apparent in the gastric and intestinal mucous membranes from 5 min to 4 hr after the injection. At 1 and 4 hr, radioactivity was discerned in the intestinal tract. At 24 hr, when the major part of radioactivity had been eliminated from the animal via the urine, the film readily showed a concentration of radioactivity in the intestinal content.

Excretion of N-*methyl*-^{14}C-3-(1-*hydroxyethyl*)*pyridinium ion*

Data on the excretion of the radioactivity from the administered compound under the various conditions are shown in Tables 2–5.

DISCUSSION

The high total excretion of radioactivity (95–99 per cent of the administered amount) in 24 hr after administration of (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide to rats (Table 2) closely parallels in many ways the similar high recovery obtained with other N-methyl-^{14}C-pyridinium compounds.[21, 22]

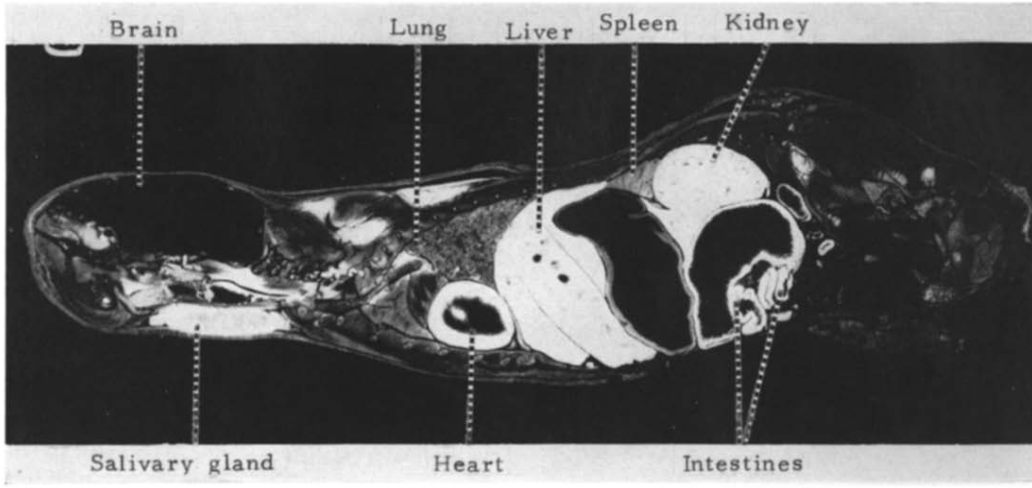

FIG. 1. Autoradiogram showing the distribution of radioactivity (light areas) in a mouse 5 min after intravenous injection of N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide. Sagittal section through the entire body of the animal. Exposure time: 52 days. Note concentration in liver, kidney, intestinal mucosa, salivary gland, and myocardium. No, or very little, radioactivity is seen in the brain

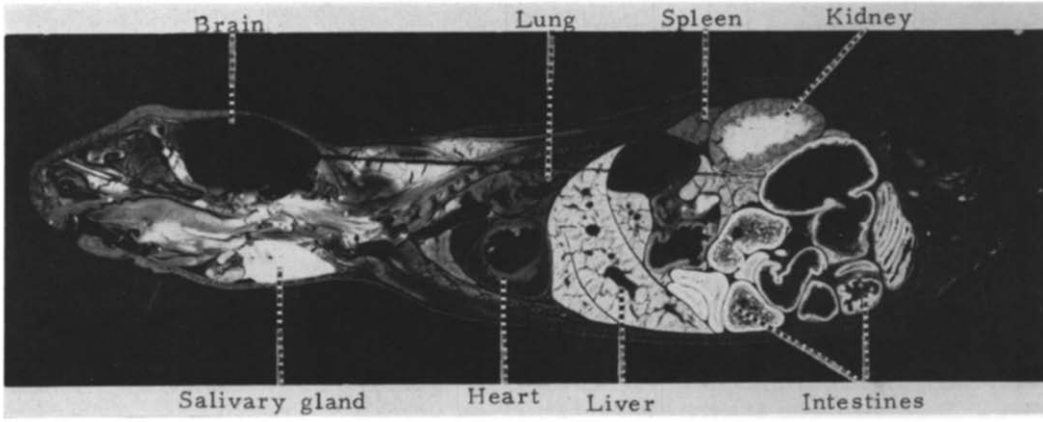

FIG. 2. Autoradiogram showing the distribution of radioactivity (light areas) in a mouse 20 min after intravenous injection of N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide. Sagittal section through the entire body of the animal. Exposure time: 52 days. Concentration is seen in the liver, salivary gland, kidney, and intestines.

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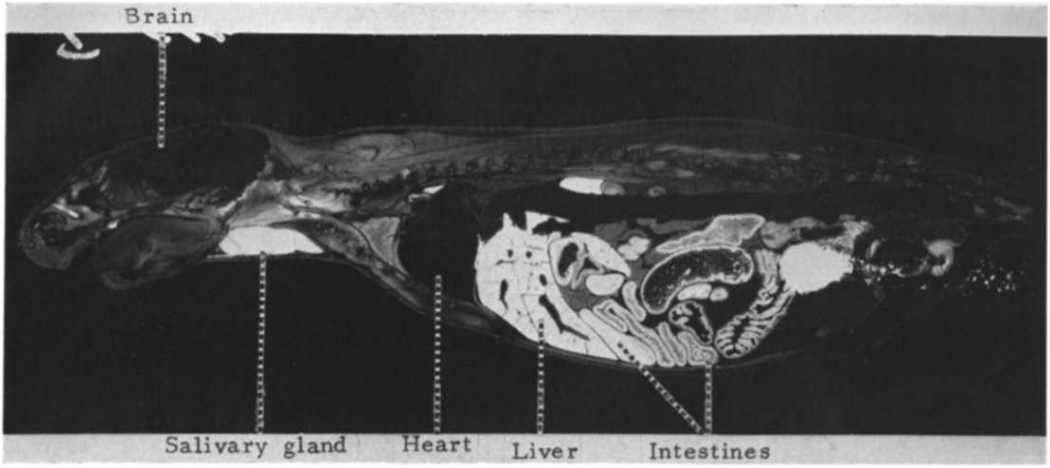

FIG. 3. Autoradiogram showing the distribution of radioactivity (light areas) in a mouse 1 hr after intravenous injection of N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide. Sagittal section through the entire body of the animal. Exposure time: 52 days. Concentration is seen in the liver, salivary gland, kidney, and intestines.

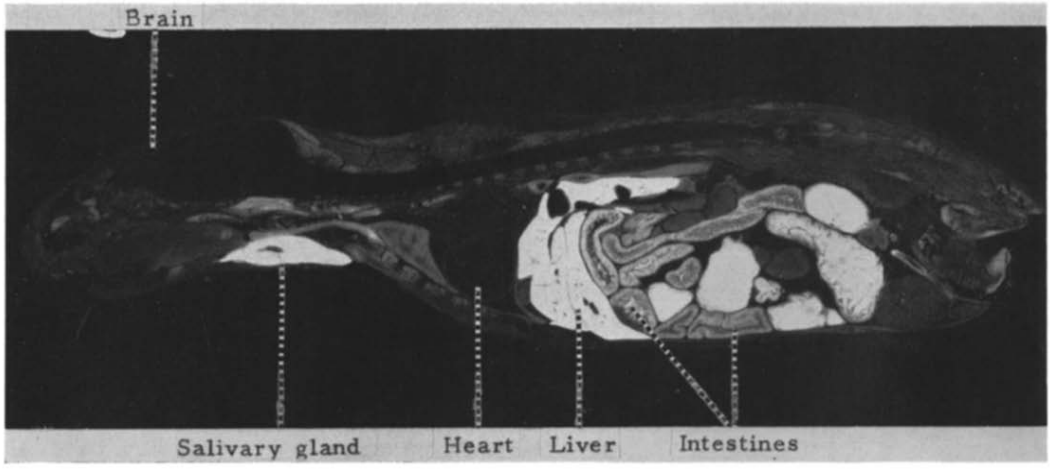

FIG. 4. Autoradiogram showing the distribution of radioactivity (light areas) in a mouse 4 hr after intravenous injection of N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide. Sagittal section through the entire body of the animal. Exposure time: 52 days. Concentration is seen in the liver, salivary gland, kidney, intestines, and intestinal content.

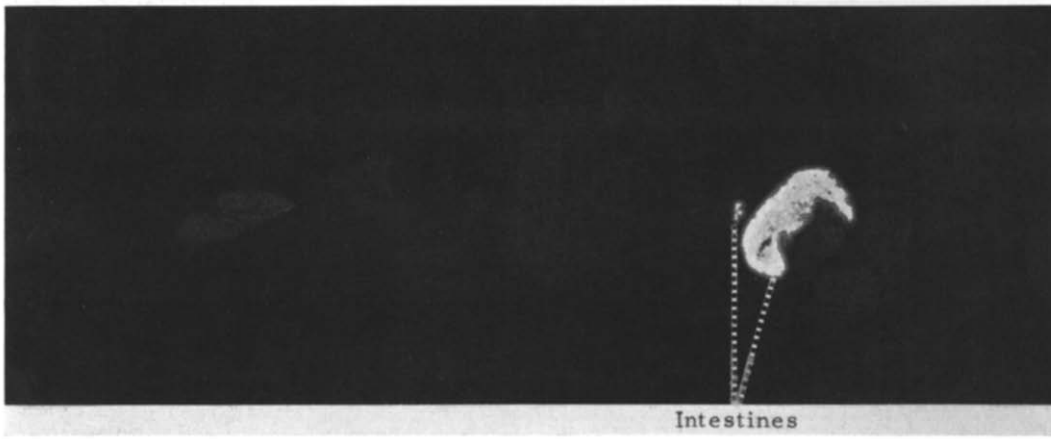

FIG. 5. Autoradiogram showing the distribution of radioactivity (light areas) in a mouse 24 hr after intravenous injection of N-methyl-^{14}C-3-(1-hydroxylethyl)pyridinium iodide. Sagittal section through the entire body of the animal. Exposure time: 52 days. Radioactivity is seen in the intestinal content

During the 24-hr period of the current study, only a small amount of radioactivity (approximately 0·1 per cent of that administered) was recovered in the respiratory carbon dioxide of rats that had received (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)-pyridinium iodide. This amount of $^{14}CO_2$ is comparable to that obtained from nicotinic acid methonium iodide and 3-pyridylacetic acid methonium iodide in the previous study,[21] and in contrast to results obtained with 3-acetylpyridine methonium iodide

TABLE 2. TWENTY-FOUR HOUR EXCRETION OF ^{14}C AFTER INTRAPERITONEAL ADMINISTRATION OF (+,−)-N-METHYL-^{14}C-3-(1-HYDROXYETHYL)PYRIDINIUM IODIDE* TO MALE ALBINO RATS

Animal weight (g)	Per cent of ^{14}C-activity recovered			
	Respiratory CO_2	Feces	Urine	Total
223	0·05	16·1	80·0	96·2
225	0·04	9·5	89·8	99·3
258	0·09	6·34	88·9	95·3

* 6·15 mg.

TABLE 3. RECOVERY OF N-METHYL-^{14}C-3-(1-HYDROXYETHYL)PYRIDINIUM ION BY ISOTOPIC DILUTION FROM 24-HOUR URINES OF RATS AND MICE AFTER ADMINISTRATION OF N-METHYL-^{14}C-3-(1-HYDROXYETHYL)PYRIDINIUM IODIDE

Animal no. and weight (g)	Dose (mg) (μc)*	Administered radioactivity in urine (%)	Urine radioactivity as administered compound† (%)
Rat 18 (223)	6·15 (6·03)	80·0	74 (77)
Rat 19 (258)	6·15 (6·03)	88·9	83 (87)
Mouse 7 (22)	0·74 (10·0)	83·7‡	101 (97)
Mouse 8 (22)	0·07 (1·0)	77·6§	93 (89)

* Compound administered intraperitoneally to rats and intravenously (via tail vein) to mice.

† Values obtained by use of carrier N-methyl-3-(1-hydroxyethyl)pyridinium iodide and (picrate) as described in Materials and Methods.

‡ Of administered radioactivity 2·2% was in the feces.

§ Of administered radioactivity 6·4% was in the feces.

TABLE 4. BILIARY EXCRETION OF ^{14}C-ACTIVITY DURING 6 HOURS AFTER ADMINISTRATION OF (+,−)-N-METHYL-^{14}C-3-(1-HYDROXYETHYL)PYRIDINIUM IODIDE* TO MALE ALBINO RATS

Animal weight (g)	Injected dose in bile (%)
316	0·42
292	0·46
270	0·47

* 0·07 mg.

where 4–8 per cent of the administered radioactivity was eliminated[21] as respiratory carbon dioxide.

In the acute toxicity studies, the lethal effects of N-methyl-3-(1-hydroxyethyl)-pyridinium iodide at high doses, in comparison with the parent alcohol, 1-(3-pyridyl)-ethanol, and its metabolic precursor, 3-acetylpyridine, tend to suggest that it might

TABLE 5. CHROMATOGRAPHIC DISTRIBUTION OF ^{14}C-ACTIVITY IN 24-HOUR URINE OF MALE RATS AFTER ADMINISTRATION OF N-METHYL-^{14}C-3-(1-HYDROXYETHYL)-PYRIDINIUM IODIDE

Animal no.	R_f* value of radioactive zone				
	Solvent A	Solvent E	Solvent F	Solvent G	Solvent H
18	0·57	†	0·76	0·74	0·20
19	†	0·10	†	0·80	†

* The values agreed (±0·08 unit) with those obtained from known solutions of the administered compound.

† Not determined.

not be entirely appropriate to call N-methyl-3-(1-hydroxyethyl)pyridinium iodide a detoxication product within the literal meaning of the term. The fact that the gross acute toxicological effects of both racemic 1-(3-pyridyl)ethanol and (−)-1-(3-pyridyl)-ethanol were the same within the limits of error of the procedures cannot be readily interpreted at this point, since no comparative metabolic studies of the two optical isomers have been performed. In an early toxicologic study,[23] the intravenous LD_{50} for 3-acetylpyridine in mice was found to be 350 mg/kg, and the corresponding value for racemic 1-(3-pyridyl)ethanol was 500 mg/kg. Various values, including an intraperitoneal lethal dose of 500 mg/kg for both 3-acetylpyridine and 1-(3-pyridyl)ethanol in rats,[24] appear in the literature. Experimental observations on the effects of niacin and other compounds in overcoming the toxic effects of 3-acetylpyridine have been previously discussed.[25–27] Our observations on the protective effects of nicotinamide against massive doses of 1-(3-pyridyl)ethanol appear to confirm and extend the results of previous workers.[10]

As pointed out in the introduction, data currently available do not permit a conclusion on the optical forms of 1-(3-pyridyl)ethanol that are involved in the metabolism of 3-acetylpyridine in the rat. In our isotopic dilution studies on the recovery of N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide from urine a similar limited interpretation prevails, since the racemic carrier may not suitably distinguish between optically active and racemic forms of the methiodide.

In the mouse, available evidence might suggest a high degree of stability of N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide. Virtually all (an average of 91–99 per cent) of the radioactivity of the urine was in the form of the administered cation, and less than 0·1 per cent of the administered radioactivity appeared as respiratory carbon dioxide. Since, however, all of the administered radioactivity was not recovered in the excretory products in each instance, metabolic alteration of the administered compound remains a distinct possibility.

The studies in the rat indicated that only approximately 80 per cent of the urinary radioactivity, after administration of (+,−)-N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide, was in the form of N-methyl-3-(1-hydroxyethyl)pyridinium ion. An accounting is required for the remaining 20 per cent of radioactivity which could presumably reside in a variety of compounds, perhaps pyridones[11] or quaternary ammonium compounds with substituents at the 3-position of the pyridine ring structurally analogous to those compounds already demonstrated as metabolites of 3-acetylpyridine in the dog.[8, 12]

Failure of N-methyl-3-(1-hydroxyethyl)pyridinium iodide to show gross manifestations of toxicity similar to those produced by 3-acetylpyridine and 1-(3-pyridyl)-ethanol can presumably be correlated with a virtual incapacity of the compound to pass the blood–brain barrier of the mouse. In its general pattern of distribution N-methyl-3-(1-hydroxyethyl)pyridinium iodide presented a picture not too different from various other quaternary ammonium compounds already studied.[28, 29]

The elimination of some of the radioactivity of intravenously administered N-methyl-^{14}C-3-(1-hydroxyethyl)pyridinium iodide in the feces of the mouse and autoradiographic evidence for its presence in the intestinal contents pose the additional problem of the mechanisms of elimination of the compound. Conceivably, the high level of radioactivity in the salivary glands may be followed by salivary secretion. Secretion at various segments in lower levels of the gastrointestinal tract may also be a factor. Consideration of the possibility of biliary secretionl ed to limited studies on the anesthetized rat (Table 4). Less than 0·5 per cent of the administered radioactivity was found in the bile during a 6-hr period; other sites of elimination, including the stomach and intestinal mucosa, deserve consideration.

Also remaining to be considered are the basic mechanisms underlying the outward signs of similarity in poisoning from single intraperitoneal injections of 3-acetylpyridine and 1-(3-pyridyl)ethanol. The data of Herken[5] and others[30] have led to the development of a thesis that 3-acetylpyridine poisoning is related to the formation of a 3-acetylpyridine analog of NAD. The analog, insofar as it has been studied with respect to coenzymatic activity in the brain, exerts a lesser catalytic effect than normal NAD. The NAD analog of 1-(3-pyridyl)ethanol has thus far been reported and studied only for activity connected with its formation *in vitro* from tissue preparations peripheral to the central nervous system. Since a similar and more substantial, if not complete, loss of activity was observed when 1-(3-pyridyl)ethanol was substituted[25, 26, 31] for nicotinamide in NAD, a common underlying mechanism may be adequate to explain some aspects of the toxicity of 1-(3-pyridyl)ethanol and 3-acetylpyridine.

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