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METABOLISM OF ETHYL DIBUNATE-^{14}C: AN ANTITUSSIVE DRUG

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Abstract—The metabolism of ethyl dibunate-^{14}C (ethyl ester of 2,7-ditertiary butylnaphthalene-4-sulfonic acid)—an antitussive agent—was studied in rats and dogs. Orally administered ethyl dibunate-^{14}C appears to be absorbed slowly from the gastrointestinal tracts of rats and dogs. Peak blood levels of ^{14}C after drug administration were found at 20 to 24 hr in the rat and at 4 to 7 hr in the dog. The hepatobiliary system plays an important role in the excretion of ethyl dibunate-^{14}C in both species, and an enterohepatic cycle was suggested for the rat. In both rat and dog, the drug is excreted mainly in feces, with small amounts being excreted in urine. Residual ^{14}C was found primarily in fat and fat-containing tissues. It is estimated that 11% to 23% of the administered drug was absorbed from the gastrointestinal tract of the rat and a minimum of 5% for the dog.

MCCOLL AND LEE[1] first reported that the ethyl ester of 2,7-ditertiary butylnaphthalene-4-sulfonic acid (ethyl dibunate) suppressed experimentally induced cough in dogs and guinea pigs. Shemano *et al.*[2, 5] have investigated the pharmacology of this drug intensively and found it to be an effective antitussive which did not produce side effects at extremely high doses (up to 5000 mg/kg p.o.).

The objectives of this study were to determine absorption, excretion, and distribution of ethyl dibunate-^{14}C (EDB-^{14}C) in tissues of rats and dogs after oral administration of the drug.

METHOD

EDB-^{14}C (specific activity 3·16 mc/m-mole,) prepared from naphthalene-4-^{14}C was used in all studies. This compound was synthesized, on request, by the Volk Radiochemical Co., Skokie, Ill. Radiopurity was verified by spectrophotometric and thin-layer chromotagraphic (TLC) methods.

Assay procedures

Blood, resuspended washed red blood cells, and solid samples such as tissues and feces (5–20 mg wet weight) were digested in 2 ml Hyamine hydroxide and prepared for counting according to the method of Herberg.[3] These materials were counted in a toluene phosphor prepared by solubilizing 0·3 g dimethyl POPOP [1,4-bis-2-(4-methyl-5-phenyloxazoyl)-benzene] and 5·0 g PPO (2,5-diphenyloxazole) in 1 liter of toluene. Plasma and bile samples (13 μliters†) were dissolved directly in 2 ml Hyamine hydroxide to which the toluene phosphor was added prior to counting. Urines were filtered through Whatman 12 filter paper. Aliquots of urine (up to 0·5 ml) were diluted to a

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† Disposable, self-filling pipettes are available from Becton, Dickinson and Co., Rutherford, N.J., that accurately (within 1%) deliver this volume.

final volume of 19 ml with *p*-dioxane phosphor consisting of 60 g naphthalene, 4 g PPO, 200 mg POPOP, 100 ml methanol, 20 ml ethylene glycol, and *p*-dioxane to make 1 liter.[4] Expiratory CO_2 was trapped in 25% aqueous monoethanolamine. Aliquots (1 ml) of the aqueous ethanolamine solution were counted in *p*-dioxane phosphor. Radioactivity measurements were performed in a Packard Tri-Carb liquid scintillation spectrometer and all values corrected by using the internal standard method.

Studies in rats and dogs

EDB-^{14}C *levels in blood.* Four male rats (150–210 g), obtained from Charles River Breeding Laboratories, were fasted overnight before use. The rats were lightly anesthetized at the time EDB-^{14}C (100 mg/kg p.o.; specific activity 1 μc/mg) was administered orally. EDB-^{14}C was solubilized in diethyl ether, diluted to a specific activity of 1 μc/mg with "cold" carrier ethyl dibunate, and dissolved in sesame oil so that on removal of the ether under vacuum, the final concentration of the drug was 10 mg/ml sesame oil. Blood, 13 μliters was taken directly from the tail vein at the time intervals shown in Fig. 1, and ^{14}C was determined.

Two conditioned pedigreed beagle dogs, a male and female weighing 9·6 kg and 9·1 kg, respectively, were fasted 24 hr before the oral administration of EDB-^{14}C (100 mg/kg, specific activity 0·1 μc/mg). The drug, suspended in sesame oil, was administered at a concentration of 50 mg/ml. The stomach tube was flushed with an additional 20 ml sesame oil. After drug administration both dogs were retained individually in metabolism cages. Water was available at all times and canned dog food was given 3 hr after the drug and once daily thereafter. Blood samples (26 μliters) were taken from the ear vein before treatment and at hourly intervals from 1 to 12 hr after treatment. Blood samples were again taken at 16, 20, 24, 30, and 48 hr after feeding. Radioactivity in blood was measured at each time interval, according to procedures described above.

In a third study, two male rats received EDB-^{14}C in sesame oil at a dose of 100 mg/kg p.o. (10 mg/ml; specific activity 1 μc/mg) and two additional male rats received EDB-^{14}C at a dose of 1000 mg/kg p.o. (50 mg/kg; specific activity 0·1 μc/mg) suspended in sesame oil. These rats were sacrificed 20 hr after treatment, by cardiac exsanguination. Radioactivity was determined in whole blood, plasma, and saline (1%) washed red blood cells diluted to original volume with saline.

Metabolism study: Pathways of EDB-^{14}C *excretion.* Four rats, two of each sex (75–190 g), were fasted overnight prior to the oral administration of EDB-^{14}C (100 mg/kg; specific activity 1 μc/mg). Each rat was individually housed in a Roth metabolism cage for a period of 4 days. Food and water were available at all times. Radioactivity in urine, feces, and expired CO_2 was measured daily.

Urine and feces were collected daily for 8 days from the two dogs used in determining ^{14}C in blood. Levels of ^{14}C in urine and feces were measured daily according to procedures described above.

Role of the hepatobiliary system. One male rat (220 g) and one male dog (9·9 kg) received the labeled drug i.p. at doses of 100 mg/kg and 5 mg/kg (specific activity 1 μc/mg) respectively. Urine and feces were collected daily over a 4-day period for the rat and over a 10-day period for the dog, and ^{14}C determined. In addition, ^{14}C in the expired CO_2 was measured daily for the rat only.

In a second study, the common bile ducts of two male rats were cannulated. These rats were given EDB-^{14}C orally (100 mg/kg; specific activity 1 μc/mg). Bile was collected over a 3-day period, and ^{14}C in bile measured daily. In an additional experiment, 5 ml radioactive bile (specific activity 0·18 μc/ml) obtained in the previous study was re-fed by stomach tube to a rat whose bile duct was also cannulated. Bile was collected for 24 hr and the ^{14}C content of the newly collected bile was measured.

Residual ^{14}C in tissues of the rat and dog at various times after oral administration of EDB-^{14}C.Tissues were taken from rats used in the determination of ^{14}C in blood and from rats used in the metabolism studies. These rats were sacrificed under ether anesthesia at 48 and 96 hr, respectively, after drug administration. The following tissues were excised, weighed, and portions of each used for the measurement of ^{14}C: intestinal tract (stomach, duodenum, jejunum, ileum, cecum, large intestines), intestinal contents, liver, kidney, spleen, testes or ovaries, brain, heart, lung, thymus adrenals, skin, and carcass. Portions of muscle and fat were also digested in Hyamine hydroxide and the ^{14}C measured.

The dogs used in the metabolism study were sacrificed 9 days after drug treatment. At autopsy, portions of each tissue, similar to those obtained in rats, were digested in Hyamine and specific activity for each tissue determined.

Absorption of EDB-^{14}C *from the gastrointestinal tract.* Estimates for EDB-^{14}C absorption from the gastrointestinal tract of rats were based on the percentage of ^{14}C found in urine, bile, and residually in the body (excluding that percentage found in intestinal contents) at 48 hr after drug administration. These data were assembled from the studies described above. Estimates of EDB-^{14}C absorption in dogs were based on the amount of ^{14}C recovered in urine over an 8-day period after the oral administration of the labeled drug.

RESULTS

EDB-^{14}C *levels in blood*

The average ^{14}C levels in blood for the four rats and the individual ^{14}C blood levels for the dogs are shown in Fig. 1 as a function of time after oral administration of EDB-^{14}C (100 mg/kg). In rats, ^{14}C was detected 4 hr after feeding with peak levels, equivalent to 19 μg EDB/ml blood, occurring at 20 to 24 hr. Small amounts of ^{14}C were still evident in blood of rats 48 hr after drug treatment.

Levels of ^{14}C were detected in the blood of dogs as early as 2 hr after drug administration (Fig. 1). Peak levels occurred at 4 hr in one dog and 7 hr in the other (equivalent to 6·2 and 8·2 μg ethyl dibunate/ml respectively; Fig. 1). These peak levels were not as high as those found in the rat and occurred earlier; the reason for this species difference is not known at the present time. Small amounts of ^{14}C were detected in the blood of dogs 48 hr after drug treatment.

A tenfold increase in the dose of EDB-^{14}C (1000 mg/kg p.o.) administered to rats produced a fourfold increase in blood ^{14}C 20 hr after feeding, a time period when peak ^{14}C levels were found with the lower dose (Table 1). Plasma contained most of the radioactivity (Table 1). These data show that blood levels of ethyl dibunate are dependent on dose.

Metabolism study: pathways of EDB-^{14}C *excretion*

Table 2 shows excretion of ^{14}C in urine and feces of rats over a 4-day period after

the oral administration of EDB-^{14}C. Urine contained an average of 2·8% of the administered ^{14}C. Most of the administered ^{14}C (approximately 90%) was recovered in feces. Expiratory carbon dioxide contained no detectable radioactivity. Approximately 75% to 85% of the administered radioactivity was excreted during the first 2 days.

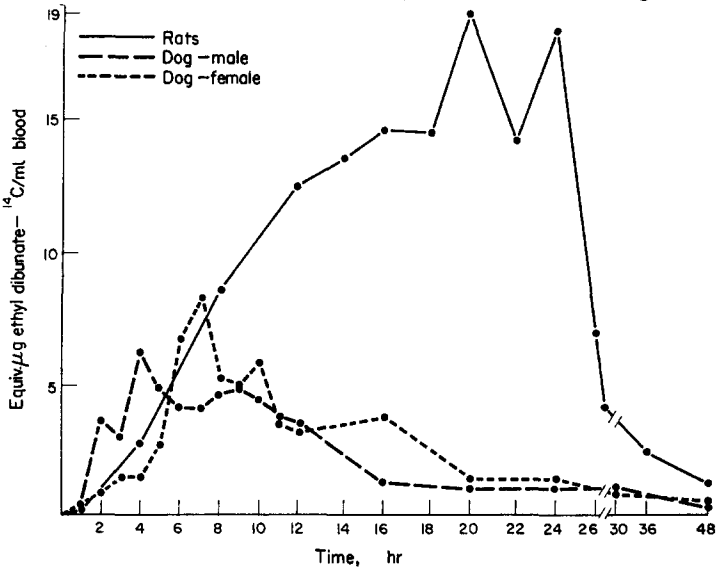

FIG. 1. Blood levels of ^{14}C, reported as μg equivalents ethyl dibunate/ml blood, in rats and dogs as a function of time after the oral feeding of ethyl dibunate-^{14}C at 100 mg/kg. The results reported for rats are based on the arithmetic mean obtained from four male rats. Individual results are reported for the dogs.

TABLE 1. LEVELS OF ^{14}C IN BLOOD, PLASMA, AND RED BLOOD CELLS OF RATS 20 HOURS AFTER ORAL ADMINISTRATION OF ETHYL DIBUNATE-^{14}C AT TWO DOSAGE LEVELS

	Dosage level, 100 mg/kg p.o.			Dosage level, 1000 mg/kg p.o.		
	Rat 1	Rat 2	Mean	Rat 1	Rat 2	Mean
	(μg equivalent ethyl dibunate/ml)					
Blood	14·45	16·45	15·45	55·10	62·97	59·04
Plasma	27·99	22·76	25·38	86·22	96·96	91·59
Red blood cells	0·72	1·04	0·88	2·50	5·01	3·76

The results in dogs were similar to those in rats. After the oral administration of the labeled drug, 4·8 and 5·9% of the administered ^{14}C appeared in urine and 78·8 and 51·6% appeared in feces over the 8-day period (Table 3). Most of the ^{14}C was excreted during the first 2 days. Small amounts of ^{14}C were still evident in urine and feces 8 days after the oral dose.

Role of the hepatobiliary system

EDB-^{14}C was given intraperitoneally to a rat and a dog to evaluate the role of the liver in the excretion of the drug. In the rat, 9 % of the ^{14}C was recovered in feces, and trace amounts (0·2%) were found in urine over the 4-day period. These low recoveries compared to those obtained after oral drug administration could be accounted for by poor absorption of drug from the peritoneum. No ^{14}C was detected in the CO_2 of

the rat. In the dog, 36% of the ^{14}C was recovered in feces and 6% in urine over the 10-day period. In both species the major pathway of excretion appears to be by way of the bile into the feces.

In those rats in which bile was collected over a 3-day period after oral drug administration, 3·6 and 7·4% of the administered ^{14}C was recovered. Of this amount, 60% was recovered during the first 24 hr. In the rat in which 5 ml of radioactive bile (specific activity 0·18 μc/ml) obtained in an earlier experiment was re-fed, the 24-hr collection of new bile contained 26% of the radioactive bile administered. These data demonstrated that ethyl dibunate or its metabolites could be recycled through the enterohepatic circulation.

TABLE 2. PER CENT RECOVERY OF ^{14}C IN URINE AND FECES OF RATS AFTER ORAL ADMINISTRATION OF ETHYL DIBUNATE-^{14}C

Time (hr)	Mean per cent recovery	
	Urine	Feces
0–24	2·10 (0·95–3·30)	43·3 (11·2–75·5)
24–48	0·37 (0·22–0·55)	37·1 (22·2–50·8)
48–72	0·25 (0·12–0·42)	6·6 (4·5– 8·7)
72–96	0·07 (0·02–0·12)	2·8 (1·8– 4·6)
Total	2·79 (1·41–3·87)	89·8 (41·6–118·7)

Values in parentheses represent the range of per cent ^{14}C recoveries for four rats.

TABLE 3. PER CENT RECOVERY OF ^{14}C IN URINE AND FECES OF DOGS AFTER ORAL ADMINISTRATION OF ETHYL DIBUNATE-^{14}C

Times (hr)	Urine		Feces	
	Male	Female	Male	Female
	(% recovery)			
0–24	1·61	2·07	24·8	10·6
24–48	0·82	1·02	29·4	21·4
48–72	0·80	0·77	10·0	8·1
72–96	0·46	0·37	4·4	3·0
96–120	0·40	0·44	3·5	1·9
120–144	0·27	0·37	2·6	3·8
144–168	0·25	0·48	1·4	1·0
168–192	0·19	0·34	2·7	1·7
Total	4·80	5·86	78·8	51·5

Residual ^{14}C in tissues of the rat and dog at various times after oral administration of EDB-^{14}C

In the rat, ^{14}C could not be detected in spleen, testes, heart, lung, thyroid, thymus, or muscle at 48 hr after EDB-^{14}C administration. Trace amounts of ^{14}C were found in stomach, kidney, and adrenal. Duodenum, jejunum, ileum, cecum, large intestine, carcass, and skin contained ^{14}C ranging from 8 to 37 μg equivalents of ethyl dibunate/g

tissue wet weight. The highest specific activities, ranging from 40 to 165 μg equivalents of ethyl dibunate/g tissue, were found in fat. Forty-eight hours after drug administration, the intestinal contents contained ^{14}C equivalent to 3·4% (range 2·9–4·4) of the administered ^{14}C. Traces of ^{14}C were still evident in fat and skin 96 hr after drug administration. The intestinal contents still contained approximately 0·5% (range 0·28–0·80) of the administered ^{14}C 96 hr after drug administration. An average of 13·9% (range 11·0–15·4) of the administered ^{14}C was found in the body of the rat 48 hr after drug treatment, which decreased to 4·8% (range 2·2–7·7) 96 hr after the drug treatment.

The distribution of residual ^{14}C in the tissues of the dog 9 days after EDB-^{14}C was similar to that found in the rat. In general, residual ^{14}C was found primarily in fat and tissue that contain large amounts of fat. No ^{14}C was found in the following tissues: aortic arch, esophagus, eye, salivary gland, uterus, testes, diaphragm, tongue, trachea, stomach, heart, spleen, bladder, kidney, lung, medulla, cerebellum, cerebrum, pituitary, or muscle taken from leg and neck. Trace amounts of ^{14}C, ranging from 3 to 40 μg equivalents of ethyl dibunate/g tissue wet weight, were found in lymph node, duodenum, jejunum, ileum, cecum, large intestine, liver, thymus, thyroid, adrenals, ovaries, and skin. The highest specific activities (ranging from 40–100 μg equivalents of ethyl dibunate/g tissue wet weight) were found in bone marrow, mammary gland, gall bladder, pancreas, and fat sampled from around the spleen, kidney, heart, and prostate. It was estimated that 1–2% of the administered ^{14}C was found residually in the dog 9 days after drug administration.

Absorption of EDB-^{14}C *from the gastrointestinal tract*

Calculation of the amount of EDB-^{14}C absorbed from the gastrointestinal tract is complicated by the finding that ethyl dibunate or its metabolites can be recycled through the enterohepatic system: however, based on the percentage of ^{14}C found in urine, bile, and residually in the body of the rat (excluding that percentage found in intestinal contents) at 48 hr after oral administration of EDB-^{14}C, a minimum of 11 to 23% of the administered ^{14}C was absorbed (Table 4). The data shown in Table 4 were assembled from the studies described above.

TABLE 4. PER CENT ABSORPTION OF ETHYL DIBUNATE-^{14}C FROM THE GASTROINTESTINAL TRACT OF THE RAT, BASED ON THE RECOVERY OF ^{14}C IN URINE, BILE, AND TISSUES, 48 HOURS AFTER ORAL DRUG ADMINISTRATION

	Mean	Range	
		Low (% Recovery)	High
Urine (4)	2·47	1·17	3·55
Bile (2)	5·07	3·28	6·85
Tissues* (3)	10·49	6·58	12·56
Total	18·03	11·03	22·97

Number of determinations in parentheses.

* Residual ^{14}C in tissues, excluding radioactivity found in intestinal contents.

Calculations based on the amount of ^{14}C found in urine of the dog over an 8-day period showed that a minimum of 5% was absorbed from the gastrointestinal tract. In all probability, the actual amount of EDB-^{14}C absorbed is significantly greater than this minimal estimate since it was shown that EDB-^{14}C or its metabolites are excreted by way of the hepatobiliary system after intraperitoneal administration of drug.

DISCUSSION

The absorption of ethyl dibunate from the gastrointestinal tract was demonstrated by the appearance of ^{14}C in blood, urine, and tissues of both rat and dog and by the appearance of ^{14}C in bile of the rat. The drug appears to be absorbed slowly, as shown by the time of first appearance of ^{14}C in blood and by the time after drug administration that peak blood levels of ^{14}C are reached. Earlier studies in rats, in which we used chemical methods for the detection of ethyl dibunate, indicated that some of the drug is absorbed by way of the lymphatic system (unpublished results). A quantitative estimate of the percentage of drug absorbed from the gastrointestinal tract is made difficult because some of the drug is excreted by way of the bile, and the ^{14}C products found in bile are partly recycled through the enterohepatobiliary system. It is estimated, however, that 11–23% cent is absorbed from the gastrointestinal tract of the rat and a minimum of 5% from the gastrointestinal tract of the dog.

In both species, ethyl dibunate or its metabolic products were preferentially found in fat and tissues that contain large amounts of fat. This, in all probability, results from the lipophilic characteristics of the drug. The appearance of small amounts of ^{14}C in urine and feces for many days after the oral administration of drug suggests that ^{14}C materials are being slowly released from these fat depots and excreted, the major pathway of excretion being by way of the feces.

Although the rat and dog appear to handle the drug similarly insofar as tissue distribution and excretion are concerned, some species difference was apparent with respect to the time after oral drug administration at which peak levels of ^{14}C occurred in blood and the concentration of ^{14}C found at those times. In rats, peak levels of ^{14}C occurred later, and the concentration of ^{14}C in blood was two to three times higher than in the dog, even though both species were given the same dose of drug (100 mg/kg). The reason for this difference is not known; however, the concentration of drug (10 mg/ml for the rat vs. 50 mg/ml for the dog) and the physical state of the drug (solution vs. suspension) could be contributing factors. These same reasons could apply to the study with rats in which a tenfold increase in dose resulted in a fourfold increase in blood ^{14}C concentration. Species differences with regard to rates of absorption and disposition of the drug may also play a contributing role.

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