Metabolism of ^{14}C-Endothall in Rats

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When ^{14}C-labeled endothall was administered orally to rats, over 90% of the radioactivity was recovered in the feces. The remainder of the radioactivity was recovered from the urine and as expired CO_2. Virtually complete recovery of the administered dose was obtained within 48 hours. The radioactivity in urine and feces was due to unchanged endothall, that present in the feces being mostly bound in some form. Small quantities of the chemical were absorbed and distributed to various tissues; however, none was detected in the fat nor excreted in the milk.

Endothall, 7-oxa-bicyclo(2,2,1)heptane-2,3-dicarboxylic acid, was first introduced in 1951 by the Sharples Co., as an experimental herbicide. Of the three isomers, the exo-cis isomer has the greatest biological activity. In addition to its use as a preemergence herbicide for the control of weeds in sugar beet, endothall recently has been adopted as a defoliating agent in cotton plants. If used in the soybean crop or as a defoliating agent in cotton, it may remain as a residue in the crops which are usually utilized for the production of vegetable oils and cattle feed.

The symptoms of endothall poisoning in mammals have been reported (Goldstein, 1952; Srensek, 1951). Both of these groups found that intravenous injection of 10 to 15 mg. per kg. in dogs and rabbits resulted in death after 60 to 130 minutes. Srensek (1951) reported that death was due to respiratory failure, while Goldstein (1952), in a later paper, demonstrated that the fatal effect of endothall was due to heart failure. Little or no information has been reported in the literature on the metabolism of endothall in animals. The present study was undertaken to obtain some information on the metabolic fate of endothall in rats following a single oral administration of the chemical.

MATERIALS AND METHODS

Experimental Animals. Adult rats of the Wistar strain, three months old, weighing from 250 to 260 grams for the male and 172 to 206 grams for the female, were used for the studies of tissue accumulation and the pattern of elimination of endothall. Two lactating females, seven months old, were used to study possible secretion of endothall in the milk.

^{14}C-endothall, labeled at carbons 1 and 2 of the ring, was synthesized by Tracerlab, with a specific activity of 1.11 mc. per mmole. The purity was greater than 98.5% by paper chromatography. A solution of ^{14}C-endothall used in the studies of tissue accumulation and elimination pattern was prepared by dissolving the radioactive endothall in 20% ethanol to a final concentration of 1 mg. per ml. For experiments with the lactating females, the ^{14}C-endothall solution was prepared by dissolving it in 10% sucrose solution to a concentration of 0.2 mg. per ml.

All rats were given a regular laboratory diet containing 5 p.p.m. of nonlabeled endothall for at least two weeks prior to the administration of radioactive endothall. Food and water were given to the rats during the entire experimental period.

Measurement of Radioactivity. Dry tissues were counted directly with a gas flow Geiger counter, and the activity was expressed as counts per minute per total organ. The levels of radioactivity in the urine and the extracts of feces were determined with a liquid scintillation spectrometer or with a Geiger counter in the usual manner. The fecal residues remaining after alcohol extraction were counted directly. The detailed method of counting and the efficiency of the instruments have been described (Khanna, 1966; Wang, 1965).

Elimination Pathway. For this study, six rats were lightly anesthetized with ether, and 1 mg. of the ^{14}C-endothall was administered orally through a stomach tube (5 mg. per kg. of body weight). Immediately after dosing, the rats were placed in the metabolic cage and the radioactivity of the expired CO_2 was monitored continuously with a flow ionization chamber and Cary Model 3810 vibrating reed electrometer. The urines and feces were collected separately, as indicated. Aliquots of the urine samples were taken for the measurement of radioactivity. Fecal samples were extracted with 50% ethanol until no more activity was found in the extracts, and the radioactivity in the extracts was measured. Dry fecal residues were counted directly in the gas flow GM counter and corrected for self-absorption factors.

Tissue Accumulation. Nine female rats were given an oral dose of 1 mg. of ^{14}C-endothall. The rats then were sacrificed at predetermined intervals from 1 to 72 hours after dosing. The various organs and tissues were carefully removed and freeze-dried in a Virtis-Roto lyophilizer. The dried samples were ground to a fine powder, aliquots of 100 mg. were placed evenly on the planchet, and the level of radioactivity was measured. The activity was corrected for self-absorption in the appropriate manner.

Lactation. To determine the possible secretion of ingested endothall in milk, and to avoid any excitation or agitation that might result from an oral administration with a stomach tube, two lactating rats were given nonradioactive endothall (0.2 mg. daily) in 10% sucrose solution by means of a medicine dropper for one week before giving birth to the young. After the pups were

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born, the mother rats were given ^{14}C-endothall (0.4 mg. daily) in 10% sucrose solution for five consecutive days. At 2, 3, 4, 6, 7, and 11 days after the first administration of radio-endothall, one pup was taken from each litter and the stomach contents, stomach, intestines, and other internal organs were analyzed for radioactivity in a manner similar to the tissue accumulation studies.

Paper Chromatography. Samples of urine and the alcohol extracts of feces, stomach, and intestine were spotted on Whatman No. 1 chromatography paper and developed at room temperature in either of the two solvents: BAW (butanol–acetic acid–water, 12:3:5, v./v./v.), or IAW (2-propanol–ammonia–water, 20:1:2, v./v./v.). The location of radioactive spots was determined by scanning the paper strips with a Packard Model 7201 chromatogram scanner.

RESULTS AND DISCUSSION

Excretion Pattern of Endothall. A study of six rats showed that an oral dose of 5 mg. per kg. of labeled endothall did not produce any gross symptoms of toxicity. Only a very small amount of C^{14} was recovered in the expired CO_2. The actual monitoring record for two rats is shown in Figure 1. The major route of excretion was in the feces, accounting for more than 90% of the administered dose. One fifth of the radioactivity in the feces could be removed by repeated extraction with 50% alcohol, and this component was shown by paper chromotography to be unchanged endothall. The remaining radioactivity presumably is due to an endothall conjugate. The level of radioactivity detected in the urine was small (5.3 to 6.8% in the first day, 0.27 to 0.57% in the second day.) Because of almost complete recoveries of radioactivity in two days (Table I), subsequent experiments were terminated after 72 hours.

Accumulation and the Rate of Elimination of Endothall in Tissues. The specific radioactivity and total accumulation in each tissue from rats sacrificed at various post-dosage times are given in Tables II and III. Analysis of the distribution data indicated that a small proportion

Figure 1. Continuous monitoring record of expired $^{14}O_2$ from rats receiving oral dose of 1 mg. of ^{14}C-endothall

2 is male rat and 3 is female

Table I. Daily Recovery of Radioactivity in CO_2, Urine, and Feces of Rats after Receiving Oral Dose of 1 Mg. ^{14}C-Endothall, in Per Cent of Administration

Rat	Sex	Body Wt.	CO_2			Urine			Feces			Total Recovery
			1	2	3	1	2	3	1	2	3	
1	♀	193	2.8	0	0	6.7	0.4	0.2	71.2	17.7	0.1	99.1
2	♂	250	2.6	0	0	6.8	0.5	0.1	67.1	17.6	0.1	94.8
3	♀	206	2.5	0	0	5.9	0.6	0	68.3	20.7	0.5	98.5
4	♂	261	2.5	0	0	5.3	0.4	0	67.3	20.1	0.1	95.7
5[a]	♀	206	2.6	0	...	5.6	0.3	...	70.6	20.5	...	97.0
6[a]	♀	172	2.6	...	...	3.4	...	...	64.5	...	...	70.5

[a] Rats 5 and 6 terminated after 48 and 24 hours, respectively.

Table II. Radioactivity in Organs of Rats Receiving Oral Dose of 1 Mg. ^{14}C-Endothall and Sacrificed at Various Times after Dosing

(1 mg. ^{14}C-endothall = 407,000 c.p.m.)

Tissues	C.P.M. per 100 Mg. of Dry Tissue								
	1 Hr.	2 Hr.	4 Hr.	6 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
Liver	242	114	114	113	57	71	42	42	0
Kidney	1,070	312	320	89	81	85	77	42	0
Heart	135	57	56	42	35	42	7	14	0
Lung	210	57	48	39	36	49	35	35	0
Spleen	71	28	28	28	28	28	14	14	0
Brain	43	35	25	18	17	7	7	14	0
Stomach	36,400	26,800	11,900	1,100	320	78	60	42	0
Intestine	2,400	2,600	6,400	4,700	4,900	1,635	960	14	0
Blood	309	96	92	56	39	28	0	0	0
Muscle	50	32	31	0	0	0	0	0	0
Fat	0	0	0	0	0	0	0	0	0

Table III. Radioactivity in Organs of Rats Receiving Oral Dose of 1 Mg. ^{14}C-Endothall and Sacrificed at Various Times after Dosing

(1 mg. ^{14}C-endothall = 407,000 c.p.m.)

Organs	C.P.M. per Organ								
	1 Hr.	2 Hr.	4 Hr.	6 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
Liver	4,600	2,460	2,280	2,010	1,310	1.260	875	780	0
Kidney	3,480	1,060	1,090	284	235	246	230	120	0
Heart	184	91	87	66	55	63	19	20	0
Lung	460	165	144	90	92	137	80	77	0
Spleen	71	42	41	29	33	28	16	13	0
Brain	104	91	65	44	42	16	18	38	0
Stomach	306,000	280,000	164,000	38,500	3,600	1.000	540	190	0
Intestine	96,000	117,000	268,000	250,000	220,000	65.000	51,500	18,500	400

of the administered dose was absorbed and transported rapidly to different tissues. Some of the absorbed material apparently was oxidized to CO_2 or excreted in the urine. With the exception of the intestine, the peak concentration in the tissues was reached 1 hour after dosing. Possibly, the maximum may have been reached before that. There was a very rapid decrease in radioactivity, and all tissues showed no detectable radioactivity at 72 hours. Excluding the stomach, the highest accumulation of endothall was found in the intestine, next was in the kidney, blood, liver, lung, heart, spleen, muscle, and brain, in that order. No radioactivity was found in the fat. Figure 2 shows the semilogarithmic plots of the rate of elimination of endothall from intestine, stomach, kidney, and liver. The rate of clearance varies in some tissues; however, those for spleen, brain, lung, heart, and liver are similar (Figure 2*D*). Some recycling could occur through absorption and elimination in the bile but it does not seem to be of significant proportions, since the levels of activity observed in the liver are not very large compared to the original dose.

Nature of the Radioactivity in Urine and the Alcohol Extracts of Feces, Intestine, and Stomach. Paper chromatography was used to determine the nature of the radioactive components. In all cases, only one radioactive spot, which coincided with radio-endothall, peak I, was observed (Table IV). Repeated extraction of feces, stomach, or intestines by 95% ethanol removed approximately only 1/5 of the total activity; the remaining radioactivity could be extracted by ethanol which contained 1%

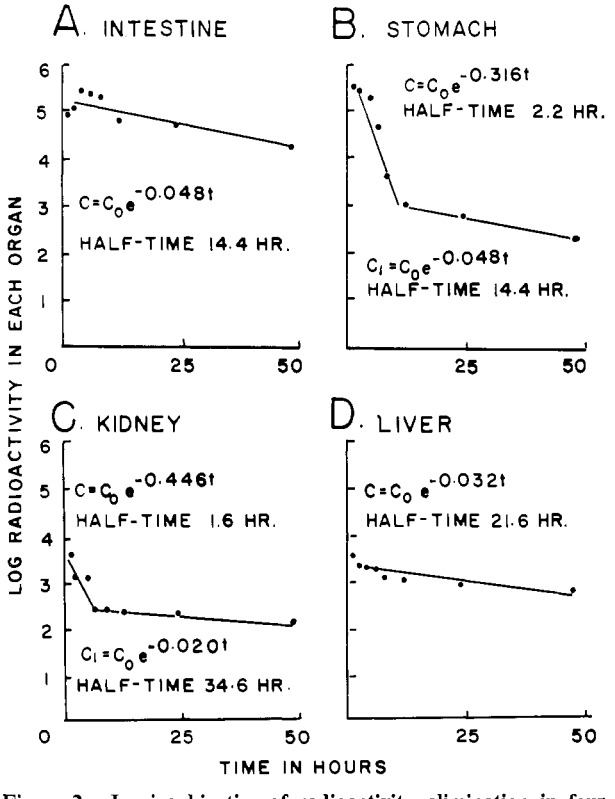

Figure 2. In vivo kinetics of radioactivity elimination in four internal organs of female rats following single oral dose of 1 mg. of ^{14}C-endothall

HCl. Paper chromatography of the 1% HCl ethanol extracts showed two radioactive peaks, II and III, which were not endothall. Further investigations revealed these components to be mono- and di-esters of the endothall which were formed during extraction. This observation was confirmed by conversion of these two radioactive compounds to endothall by acid or base hydrolysis. Also, if the alcohol-extracted samples were extracted with 1% aqueous HCl, only unchanged endothall was obtained. This indicates that esterification of endothall occurred with ethanol in the presence of mineral acid.

Table IV. R_f Values of Radioactive Peaks in IAW and BAW Solvent Systems

Peak	R_f Values IAW	BAW
I (Endothall)	0.00	0.65
II[a] Monoester of endothall	0.35	0.82
III[b] Diester of endothall	0.87	0.87

[a] Identified by direct synthesis. One mole of endothall reacting with 1 mole of ethanol in presence of mineral acid at 100° C. for three hours forms endothall monoester.
[b] When ethanol is in excess, both mono- and diesters ofen dothall are formed.

Milk Pathway. Analysis of stomach contents and tissues of 12 pups of various ages showed no radioactivity, suggesting that endothall was not secreted in the milk of a lactating female rat. The fact that no endothall was observed in the fat or excreted in the milk would be consistent with the hydrophylic nature of this compound.

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