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The Fate of Ethyltin and Diethyltin Derivatives in the Rat

By J. W. BRIDGES, D. S. DAVIES and R. T. WILLIAMS
Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2

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1. Ethyltin trichloride does not appear to be metabolized by the rat. When given orally excretion occurs almost entirely in the faeces, and when given intraperitoneally it occurs exclusively in the urine. Biliary excretion is almost negligible. 2. Di[1-^{14}C]ethyltin dichloride has been synthesized. When given intraperitoneally it is excreted in the urine and faeces in the ratio about 1:2. Both the urine and faeces contain ethyltin^{3+} and diethyltin^{2+}. Diethyltin is also excreted extensively in the bile. Di[^{14}C]ethyltin is not converted into $^{14}CO_2$ in the rat. 3. About 50% of the injected diethyltin dichloride is de-ethylated to ethyltin^{3+}. Since ethyltin and diethyltin are found in the urine and faeces after intraperitoneal injection of diethyltin dichloride and since only diethyltin is excreted in the bile, then the de-ethylation of diethyltin occurs in the body tissues and the gut. 4. The conversion of diethyltin into ethyltin has been demonstrated in a preparation of rat caecal contents, but not in liver homogenates. 5. The dealkylation of diethyltin^{2+} to ethyltin^{3+} in the rat is discussed and it is suggested that the ethyl group is lost as ethane.

Organotin compounds, some of which are highly toxic, have found use in medicine, agriculture, ship cleaning and industry as bactericides, anthelminthics, insecticides, fungicides, plastic stabilizers etc. (see Johnson, 1962; Barnes & Stoner, 1959). There are four classes of alkyltins, which can be represented by the general formulae $RSnX_3$, R_2SnX_2, R_3SnX and R_4Sn, where R is the alkyl group and X is any radical not linked to tin through a carbon atom. These four classes appear to have distinct biological properties, which depend, within any class, more on the nature of the R group than on that of X. As a group, the trialkyltins appear to be more toxic than the corresponding dialkyltins, which in turn appear to be more toxic than the monoalkyltins (Barnes & Stoner, 1959). Tetraalkyltins are less toxic than the corresponding trialkyltins, but the symptoms of poisoning, although delayed, are the same. It has been shown that tetraethyltin is dealkylated *in vivo* in the rat to triethyltin (Cremer, 1958) and it appears that, although tetraethyltin is probably biologically inactive as such, it exerts toxic effects through conversion in the liver into triethyltin. Little is known about the metabolic transformations of other alkyltins and there appears to be no published work on the metabolism of mono- and di-alkyltins.

It is shown here that monoethyltin is not metabolized in the rat but that diethyltin is partially metabolized and converted into monoethyltin. These compounds were apparently not degraded to inorganic tin. It is suggested that the ethyl group removed *in vivo* from diethyltin^{2+} may appear as ethane. These results have been briefly reported (Bridges, Davies & Williams, 1965; Davies & Williams, 1966).

MATERIALS AND METHODS

Materials

Tin, $SnCl_4$ (Hopkin and Williams Ltd., Chadwell Heath, Essex) and tetraethyltin (b.p. 65°/12mm.; Kirby Fine Chemicals, Liverpool) were purchased. Diethyltin oxide was a gift from Salsbury Laboratories, Charles City, Iowa, U.S.A.

Ethyltin and its derivatives. Ethyltin trichloride was prepared from tetraethyltin by the method of Neumann & Burkhardt (1963). The product obtained in this way was a colourless liquid, b.p. 68°/6mm., which fumed slightly in air, and was about 90% pure. It was shown chromatographically to contain diethyltin. It was purified through the sulphide. A solution of $(NH_4)_2S$ (14ml.; 16%, w/v) (Hopkin and Williams Ltd.) was added to an aqueous solution of ethyltin trichloride (2ml. in 10ml. of water). The solution was acidified with 2N-HCl and ethyltin sulphide was precipitated as a white powder. The precipitate (1·5g.) was washed with water followed by ethanol and dried. It was purified by dissolving in hot dioxan and then precipitating it with water. It did not melt or decompose on heating to 320° and from molecular-weight determinations and elementary analysis appeared to be a *dimer of bis(ethyltin) trisulphide* [Found: C, 12·3; H, 2·6; S, 24·6%; mol.wt. (osmometer), 770. $(C_4H_{10}S_3Sn_2)_2$ requires C, 12·1; H, 2·6; S, 24·35%; mol.wt. 783·4].

Ethyltin trichloride (2ml.) in ether (10ml.) was added to

an ethereal solution (10ml.) of 2,2'-bipyridyl (2g.). A white precipitate separated, which was filtered, washed with ether and then recrystallized from a large volume of hot butan-1-ol to give a white fluffy powder (yield, 85%). This appeared to be the complex *ethyltin trichloride–2,2'-bipyridyl*, m.p. 226·5° (Found: C, 35·1; H, 3·2; Cl, 25·9; N, 6·8%. $C_{12}H_{13}Cl_3N_2$ requires C, 35·0; H, 3·3; Cl, 25·85; N, 6·75%).

Purification of ethyltin was achieved through the sulphide, since like inorganic tin it forms a sulphide insoluble in water. Impure ethyltin trichloride was converted into the pure trisulphide as above. The trisulphide (2g.) was suspended in ether (20ml.) and the solution saturated with dry HCl until no more H_2S was evolved. The solution was evaporated and the residual liquid distilled *in vacuo*. The ethyltin trichloride was a colourless liquid, b.p. 76°/12mm. Samples of pure ethyltin trichloride were also obtained as gifts from Dr N. P. Neumann, University of Giessen, Germany, and Dr J. G. A. Luijten, Institute of Chemistry, Utrecht, The Netherlands.

Diethyltin and its derivatives. Dry diethyltin oxide (10g.) was suspended in boiling benzene (200ml.) and anhydrous HBr gas passed through the solution. The water produced was removed by continuous azeotropic distillation and collected in a Dean–Stark trap. The HBr was passed into the solution until the calculated amount of water for the formation of diethyltin dibromide from the oxide had been collected (about 3·5hr.). The solvent was removed and diethyltin dibromide (m.p. 63°) was recrystallized from light petroleum (b.p. 100–120°). Diethyltin dichloride (m.p. 85°) was prepared similarly, with HCl instead of HBr.

The complex, diethyltin dichloride–2,2'-bipyridyl, m.p. 200°, after recrystallization from benzene, was prepared by the method of Alleston & Davies (1962), who give m.p. 200–201°.

Tetra[1-^{14}C]*ethyltin.* [1-^{14}C]Ethyl iodide (64·5m-moles or 8·2g.; 15·5μc/m-mole) (The Radiochemical Centre, Amersham, Bucks.) in dry ether (30ml.) was added to magnesium turnings (58·5m-moles or 1·42g.) at such a rate as to maintain gentle refluxing. The mixture was boiled gently for 30min. and then cooled in ice. Then $SnCl_4$ (13·2m-moles or 3·5g.) in ether (10ml.) was added with vigorous stirring. The mixture was then gently boiled for 1hr. and cooled once more. During the next 20min. the ether was distilled off and the temperature at the centre of the reaction mixture allowed to rise to 65° to remove the last traces of ether. The mixture was cooled and the ether distilled off, re-added and the whole was treated with ice-cold water (15ml.) followed by 10% (w/v) HCl (20ml.). The ether layer was separated, dried over anhydrous $CaCl_2$ and the ether distilled. The crude tetra[^{14}C]ethyltin was distilled under vacuum on a water bath at 65°/12mm. to give 1·71g. of tetra[1-^{14}C]ethyltin, b.p. 65°/12mm., of specific activity 0·23μc/mg. and radiochemical yield 40%.

Di[1-^{14}C]*ethyltin.* Tetra[1-^{14}C]ethyltin (7·3m-moles or 1·71g.) and $SnCl_4$ (7·3m-moles or 1·9g.) were heated together at 210° for 1hr. (Luijten & van der Kerk, 1955). The diethyltin dichloride formed was recrystallized from light petroleum (b.p. 100–120°). The di[1-^{14}C]ethyltin dichloride (70% yield) had m.p. 85° (literature m.p. 84·5–86°) and specific activity 0·11μc/mg.

Animals

Female Wistar albino rats (wt. 190–250g.) were used and these had free access to food (rat diet 41B; J. Rank Ltd., Deptford, London) and water. Biliary cannulation of rats was carried out as described by Abou-El-Makarem, Millburn, Smith & Williams (1967).

Administration of compounds

Ethyltin trichloride was administered to rats in aqueous solution, with 0·5ml. of water for intraperitoneal injection and 1ml. for oral dosing. Diethyltin dichloride was administered similarly. With di[1-^{14}C]ethyltin dichloride the amount of ^{14}C injected was determined by counting a series of sample deliveries from the syringe used.

Determination of tin and its compounds

Determination of total tin. Urine, faecal homogenates or tissue homogenates (5–10ml.) in narrow-necked conical flasks were treated with conc. H_2SO_4 (3ml.) followed quickly by excess of conc. HNO_3 (15ml.). The flasks were heated until white fumes of SO_3 appeared. If charring occurred, the mixture was allowed to cool slightly, a few drops of H_2O_2 (100vol.) were added and the mixture was gently warmed again. Addition of H_2O_2 and heating was repeated until colourless solutions were obtained on heating until fumes of SO_3 appeared. The sides of the flask were washed down with a little water and heating to white fumes repeated. Then the flasks were cooled, water (7ml.) was added and the contents were transferred to 50ml. volumetric flasks for colour development with dithiol as described by Farnsworth & Pekola (1959). With large amounts of faeces a white insoluble compound sometimes appeared and this was filtered at the stage of transfer to the volumetric flask. The red colour was measured at 530mμ in a Unicam SP.600 spectrophotometer. The limit of sensitivity was 5μg. of tin/ml. of sample.

Fluorimetric determination of tin and ethyltin. Sn^{4+} forms a fluorescent complex with flavonol (3-hydroxyflavone) in dil. H_2SO_4 (Coyle & White, 1957). We have found that ethyltin trichloride, ethyltin tribromide, butyltin trichloride and phenyltin tri-iodide also fluoresce under these conditions, but diethyltin dichloride even in 100-fold excess does not fluoresce, nor does it interfere with the fluorescence of ethyltin. Thus small amounts of monoethyltin can be accurately estimated in the presence of excess of diethyltin. Quadrivalent tin can be estimated similarly.

A stock aqueous solution of ethyltin trichloride (5mg./ml.) was found to be stable for several weeks. The solution was diluted with water to 25μg./ml. for routine purposes but at this strength the ethyltin was unstable if kept. A solution of 0·01% flavonol (Eastman Kodak Co., Rochester, N.Y., U.S.A.) was made up in aq. 90% (v/v) ethanol. *NN*-Dimethylformamide was used as purchased (Hopkin and Williams Ltd.). The standard curve was prepared by adding 0·2–2ml. of ethyltin trichloride solution (25μg./ml.) to 10ml. volumetric flasks followed by 1ml. each of dimethylformamide and N-H_2SO_4. To this mixture 2ml. of 0·01% flavonol solution was added and, after mixing, the solution was made up to 10ml. with water. After standing 15min. the fluorescence intensity was measured at $\lambda_{max.}$ 445mμ ($\lambda_{exc.}$ 395mμ) in an Aminco–Bowman spectrophotofluorimeter with a Corning glass filter (no. 3–73) with cut-off at 450mμ.

If Sn^{4+} or ethyltin is estimated in urine or bile then allowance must be made for the ability of phosphate ions to

quench the flavonol–tin fluorescence. This is done by means of an internal standard. The fluorescence of the unknown sample, measured as above, was determined and also of the unknown containing a known amount of added ethyltin trichloride. By a simple calculation, the quenching, which on occasions reached 60%, can be allowed for. The recoveries of ethyltin trichloride added to bile or urine (0·5–20 μg./ml.) were 98±2%.

Colorimetric determination of diethyltin dichloride. The dithizone method of Aldridge & Cremer (1957) was used. This reagent gives an orange complex ($\lambda_{max.}$ 510mμ) with diethyltin dichloride in borate buffer, pH 8·4. Under these conditions ethyltin trichloride and $SnCl_4$ produce no colour. This method was used for diethyltin^{2+} in bile but was unreliable for urine. Diethyltin^{2+} in urine was estimated radiochemically (see below). The recoveries of diethyltin dichloride added to bile (20–200 μg./ml.) by the colorimetric method was 96±5%.

Radiochemical techniques

A Packard Tri-Carb scintillation spectrometer (model 3214) was used to determine ^{14}C at 0°. The liquid scintillators used were: a 'toluene' system for non-aqueous samples, 2,5-diphenyloxazole (5g.) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0·3g.) in toluene (1l.); a 'dioxan' system for aqueous samples, naphthalene (60g.), 2,5-diphenyloxazole (4g.), 1,4-bis-(5-phenyloxazol-2-yl)benzene (0·2g.), methanol (100ml.) and ethylene glycol (20ml.) in dioxan, added to make 1l.; and a 'gel' system for heterogeneous samples, e.g. faecal and tissue homogenates, insoluble compounds; a 5% suspension of Cab-O-Sil (thixotropic gelling agent; Packard Instrument Co.) in the 'dioxan' scintillator.

Preparation of samples. Urine (1–2ml.) and bile (0·2–2ml.) were counted in 'dioxan' scintillator (15ml.), and urine (3–6ml.) and aqueous faecal and tissue homogenates (1–2ml.) in 'gel' scintillator (15ml.). Tissues (1g.) were dissolved in Hyamine (10ml.; Packard Instrument Co., Wembley, Middx.) and counted in 'toluene' scintillator, or in 40% (w/v) NaOH and counted in 'gel' scintillator. Whole rats (wt. approx. 200g.) were dissolved in 40% (w/v) NaOH (150ml.) and ethanol (50ml.) by heating at 40° for 24hr. and samples were counted in 'gel' scintillator. The recovery of known amounts of ^{14}C (0·2 μc) given to rats dissolved in this way was 89–95%. Counting efficiencies were determined by the channels-ratio method.

Isotope-dilution procedures. (*a*) Ethyltin trichloride. The carrier (1–2g.) was added to the sample (1–5ml.) of urine, bile or faecal homogenate and an excess of $(NH_4)_2S$ solution (approx. 15ml.; 16%, w/v) added. The mixture was acidified with 2N-HCl, and the precipitate of ethyltin sulphide was collected and washed with water, ethanol and finally ether. It was purified by dissolving in hot dioxan, precipitated hot by the addition of water, filtered and counted. Any diethyltin sulphide (m.p. 24°) would be liquid in hot dioxan and would not be retained on filtration. The ethyltin sulphide was now dissolved in ether saturated with HCl and to this 2,2′-bipyridyl in ether (2g./10ml.) was added. The ethyltin trichloride–2,2′-bipyridyl complex that separated was isolated and recrystallized to constant specific activity from butan-1-ol. In every case in this work the specific activity of the sulphide agreed with that of the bipyridyl complex formed from it.

(*b*) Diethyltin dichloride. The carrier (1–2g.) was added to urine, bile or faecal homogenate (1–5ml.), and the mixture acidified with 5N-HCl. The diethyltin dichloride was then extracted with ether (approx. 5vol.). The ether was evaporated and the crystalline residue recrystallized to constant activity from light petroleum (b.p. 100–120°). It was then converted into its 2,2′-bipyridyl complex, which was recrystallized to constant specific activity from benzene.

Incubation with rat caecal contents. Fresh caecal contents from two rats were suspended in 0·14M-phosphate buffer, pH 7 (100ml.), and filtered through one layer of cheese cloth (Booth & Williams, 1963). Two flasks each containing 25ml. of the filtrate and 12·55mg. of di[1-^{14}C]ethyltin dichloride and a third flask containing 25ml. of 0·14M-phosphate buffer, pH 7, and 12·55mg. of di[1-^{14}C]ethyltin dichloride were incubated for 30hr. at 37°. The contents of the flasks were agitated throughout by a continuous stream of N_2 gas. Samples from each flask were analysed for ^{14}C and, by isotope dilution, for ethyltin and diethyltin.

RESULTS

Ethyltin trichloride. Table 1 shows that when ethyltin trichloride (25mg./kg.) is given to rats orally it is poorly absorbed and most of it (92% of the dose) is excreted in the faeces in 2 days, only about 1–2% appearing in the urine. When given by intraperitoneal injection (12·7mg./kg.) it is excreted exclusively in the urine, about 73% of the dose being accounted for in 3 days. When given intraperitoneally to biliary-cannulated rats, ethyltin trichloride is again almost entirely excreted in the urine, only small quantities appearing in the bile. The methods of estimating tin, i.e. fluorimetric for urine and colorimetric for faeces, do not distinguish between inorganic tin and ethyltin, but we have reason to believe that little ethyltin is metabolized to inorganic tin. Inorganic tin forms a red complex with toluene-3,4-dithiol in the presence of thioglycollic acid, which reduces stannic tin to the stannous form. Under the same conditions ethyltin does not form a coloured complex. Urine from rats treated with ethyltin trichloride was examined for inorganic tin by this colour test, but the test was consistently negative in urine from normal and biliary-cannulated rats receiving ethyltin trichloride orally or intraperitoneally. Taking into consideration the sensitivity of this colour test under the conditions of the experiments, we conclude that, if ethyltin is converted into inorganic tin, then the amount of inorganic tin is less than 10% of the dose.

Diethyltin dichloride. Table 2 shows the distribution of ^{14}C in the excreta of rats receiving, by intraperitoneal injection, about 2mg. each of di[1-^{14}C]-ethyltin dichloride. No ^{14}C was excreted in the expired air as carbon dioxide. About 40% of the dose was excreted in the faeces, 22% in the urine and 5% was left in the carcass in 6 days after dosing. The total ^{14}C accounted for was 67% or about two-thirds of the dose, and it appears that about

Table 1. *Routes of excretion of ethyltin trichloride in rats*

Three rats (nos. 1, 2 and 3) were given orally 5·13 mg. of ethyltin trichloride each (about 25 mg./kg.) dissolved in water; three rats (nos. 4, 5 and 6) were given by intraperitoneal injection 2·5 mg. of ethyltin trichloride each (about 12·7 mg./kg.) in water; three biliary-cannulated rats (nos. 7, 8 and 9) were each given 2·5 mg. of ethyltin trichloride intraperitoneally. Urine, faeces and bile were collected; the urine and bile were analysed for tin or ethyltin fluorimetrically and the faeces colorimetrically. Average values with ranges in parentheses are given. These methods do not distinguish between ethyltin and inorganic tin.

	% of dose excreted as ethyltin/tin					
Rats	1, 2 and 3		4, 5 and 6		7, 8 and 9	
Route of administration ...	Oral		Intraperitoneal		Intraperitoneal	
Day	In urine	In faeces	In urine	In faeces	In urine	In bile
1	0·8 (0·7–0·9)	56 (46–66)	58 (51–69)	0	75 (61–83)	< 3
2	0·4 (0·2–0·8)	36 (30–45)	10 (8–11)	0	7 (2–10)	< 1
3	0·0	0	5 (4–5)	0	—	
Total	1·2 (1–2)	92 (89–96)	73 (66–82)	0	82 (69–93)	
Total excreted	93		73		82	

Table 2. *Excretion of* ^{14}C *by rats given di*[1-^{14}C]*ethyltin dichloride*

Three rats (nos. 10, 11 and 12) were given 1·98 mg. each of di[1-^{14}C]ethyltin dichloride (0·11 μc/mg.) by intraperitoneal injection in water (about 10 mg./kg.). Expired air was collected for 3 days and urine and faeces were collected for 6 days. These were analysed for ^{14}C. After 6 days the animals were killed and the ^{14}C in the carcasses was determined. Average values with ranges in parentheses are quoted.

	% of dose of ^{14}C				
Day	In urine	In faeces	In expired air as CO_2	In carcass	Total
1	13 (6–18)	18 (13–22)	0	—	
2	5 (4–7)	13 (7–19)	0	—	
3	2 (1–2)	7 (4–11)	0	—	
4	1 (0·2–1·0)	0	—	—	
5	0·4 (0·3–0·4)	0	—	—	
6	0·3 (0·1–0·4)	0	—	5 (2–8)	
Total	22 (16–26)	38 (29–51)	0	5 (2–8)	65 (54–71)

Table 3. *Excretion of tin and* ^{14}C *by rats given di*[1-^{14}C]*ethyltin dichloride*

Three rats (nos. 13, 14 and 15) were each injected intraperitoneally with 2·08 mg. of di[1-^{14}C]ethyltin dichloride in water. ^{14}C and tin were determined in the urine and faeces collected for 92 hr. after dosing.

		% of ^{14}C			% of tin		
Rat no.	Dose (mg./kg.)	In urine	In faeces	Total	In urine	In faeces	Total
13	9·5	27	29	56	41	59	100
14	8·5	15	35	50	20	73	93
15	10·6	16	33	49	33	59	92
Average values		19	32	52	31	64	95

one-third of the ^{14}C administered has been lost in some way. The experiment was repeated and both ^{14}C and total tin in the excreta were determined. As shown in Table 3, the recovery of ^{14}C and total tin do not correspond. In this experiment an average of 52% (32% in the faeces and 19% in the urine) of the administered ^{14}C was recovered, but 95% (64% in the faeces and 31% in the urine) of the administered tin was recovered in 92 hr. This experiment suggested that diethyltin was being

Table 4. *Monoethyltin and diethyltin in the urine and faeces of rats receiving di[1-^{14}C]ethyltin dichloride*

Three rats were injected intraperitoneally with an aqueous solution of di[1-^{14}C]ethyltin dichloride (10mg./kg.; 0·11μc/mg.). Urine and faeces were collected for 72hr. and analysed for ^{14}C and for monoethyltin and diethyltin by isotope dilution.

	% of dose of ^{14}C excreted					
	As monoethyltin		As diethyltin		Sum of columns	
Rat no.	Calc. as ^{14}C	Calc. as Sn	Calc. as ^{14}C or Sn	Total ^{14}C found	2+4 (^{14}C)	3+4 (Sn)
In urine						
16	11·9	23·8	8·6	21	20·5	32·4
17	18·5	37·0	0·3	20	18·8	37·3
18	16·0	32·0	5·7	21	21·7	37·7
Average	15·5	30·9	4·9	21	20·3	35·8
In faeces						
16	10·7	21·4	9·5	19	20·2	30·9
17	21·6	43·2	6·6	30	28·2	49·8
18	16·2	32·4	14·6	29	30·2	47·0
Average	16·2	32·3	10·2	26	26·2	42·6
In urine and faeces						
Average values	31·7	63·2	15·1	47	46·5	78·4

Table 5. *Biliary excretion of diethyltin dichloride in rats*

Each biliary-cannulated rat was injected intraperitoneally with 2mg. of diethyltin dichloride (about 10mg./kg.) in water. Bile was collected for 48hr. The ^{14}C-labelled compound was used with rats 19, 20, 21, 25, 26 and 27 and ^{14}C determined. Non-radioactive diethyltin was given to rats 22, 23 and 24 and the diethyltin determined colorimetrically. Ethyltin and diethyltin were determined by isotope dilution.

	% of dose excreted in 48hr.					
Rat no.	In bile	In urine	In faeces	Total	As ethyltin	As diethyltin
19	56	10	1	66	—	—
20	47	33	1	80	—	—
21	74	5	1	79	—	—
22	42	—	—	—	—	—
23	52	—	—	—	—	—
24	57	—	—	—	—	—
25	74	—	—	—	0·8	80
26	65	—	—	—	0	64·2
27	42	—	—	—	0	40·1
Average	56·5					

dealkylated in the rat. The urine and faeces were therefore examined by isotope dilution for monoethyltin and diethyltin. The results shown in Table 4 indicate that both monoethyltin and diethyltin occur in the urine and faeces. Table 4 shows that, in 72hr. after dosing, an average of about 78% of the dose is excreted (36% in urine and 43% in the faeces) when calculated on the tin dose and 46% (20% in urine and 26% in faeces) when calculated on the ^{14}C dose. There is, in fact, a considerable dealkylation of diethyltin to monoethyltin. In the urine an average of 31% of the dose occurs as monoethyltin and 5% as diethyltin, whereas in the faeces 32% occurs as monoethyltin and 10% as diethyltin. In another experiment monoethyltin was estimated fluorimetrically with flavonol, which does not fluoresce with diethyltin. Six rats were injected with non-radioactive diethyltin dichloride (10mg./kg.) and the monoethyltin was estimated fluorimetrically in the urine collected for 3 days. The individual values found for these rats were 64, 54, 52, 46, 20, 15 and

Table 6. *Incubation of di*[1-^{14}C]*ethyltin dichloride with rat caecal contents*

Solutions *A* and *B* contained a rat caecal preparation (see the Materials and Methods section) in 0·14 M-phosphate buffer, pH 7 (25 ml.), and solution *C* was 0·14 M-phosphate buffer, pH 7 (25 ml.). Di[1-^{14}C]ethyltin dichloride (12·55 mg.) was added to each, and the mixtures were incubated at 37° for 30 hr. with continuous agitation with a stream of N_2. Samples were analysed for ^{14}C. Solutions *A* and *B* were combined and analysed for ethyltin and diethyltin by isotope dilution. The diethyltin in solutions *A* + *B* initially was 101·3 μmoles. After 30 hr. incubation the diethyltin in solutions *A* + *B*, as determined by isotope dilution, was 88·2 μmoles. The diethyltin lost was therefore 13·1 μmoles. The monoethyltin formed as determined by isotope dilution was 12·8 μmoles.

Solution	10^{-6} × Radioactivity (disintegrations/min.) Initial	Final	Loss (%)
A	2·643	2·48	6·2
B	2·643	2·46	6·9
C	2·643	2·64	0·1

7·5 (average 37%), the first value being given by a rat yielding a high urine volume and the last by a rat with a low urine volume. The value of 31%, obtained by isotope dilution (Table 4), is within this range. Colour tests on the urine suggested that little or no inorganic tin was excreted.

The biliary excretion of diethyltin dichloride was also examined in the rat. Table 5 shows that the average amount of an intraperitoneal dose (10 mg./kg.) of di[1-^{14}C]ethyltin dichloride excreted in the bile in nine rats is 57% (range 42–74%). In three of these rats the ethyltin and diethyltin in the bile were determined by isotope dilution. A small amount (0·8%) of monoethyltin was found in the bile of one rat, but, as Table 5 shows, the biliary material is almost exclusively diethyltin. In three biliary-cannulated rats given non-radioactive diethyltin dichloride (10 mg./kg.), the diethyltin in the bile collected for 48 hr. was determined colorimetrically by the dithizone method and the proportion of the dose in the bile as diethyltin was found to be 42, 52 and 57%. These results suggest that about 50% of the injected diethyltin dichloride is excreted in the bile as diethyltin. Since it has been shown already that very little injected monoethyltin is excreted in the bile (see Table 1), then monoethyltin formed in the body from diethyltin would not be expected to appear in the bile, and this is what has been found.

Dealkylation of diethyltin. Table 4 shows that, after the injection of diethyltin, monoethyltin occurs both in the urine and in the faeces. Further, Table 5 shows that only diethyltin is excreted in the bile, and Table 1 shows that injected monoethyltin does not appear in the faeces, nor is it absorbed from oral dosing. These findings suggest that diethyltin is dealkylated both in the body tissues and in the gut of the rat. Di[1-^{14}C]ethyltin dichloride was therefore incubated with a preparation of rat caecal contents (Booth & Williams, 1963) and the loss of ^{14}C and the appearance of monoethyltin were estimated. Table 6 shows the result of such an experiment, in which a loss of radioactivity corresponding to 13·1 μmoles of diethyltin and the formation 12·8 μmoles of monoethyltin, as determined by isotope dilution, occurred in 30 hr. This experiment shows that the diethyltin can be slowly dealkylated to monoethyltin in the caecal contents of the rat. Various attempts (molecular sieve; activated charcoal) were made to trap the radioactivity lost (which we suggest may be ethane) and identify it, but without success.

DISCUSSION

The absorption and excretion of ethyltin trichloride in the rat is similar to that of inorganic tin (see Barnes & Stoner, 1959), for on oral administration it is hardly absorbed at all and on intraperitoneal injection it does not get into the gut via the bile or gut wall and is entirely excreted in the urine. Many of the chemical properties of ethyltin are approaching those of inorganic tin and it appears that this is true also as far as transport across the gut wall is concerned. Although it could not be proved unequivocally with the techniques used, ethyltin did not appear to be metabolized.

The mechanism of the dealkylation of diethyltin is not clear since the [^{14}C]ethyl group removed did not appear in the expired air as $^{14}CO_2$ as might be expected. Various trapping systems were employed, and if the group had been oxidized and released as ethanol or acetaldehyde it would have been detected. It is therefore suggested that the ethyl group may be liberated as ethane. There are theoretical arguments to support this view. If it is assumed that the biological oxidation proceeds by a nucleophilic mechanism, then an attack on the Sn–C bond would be directed towards the tin atom rather than the carbon. In the dealkylation of

N- and *O*-ethers, on the other hand, nucleophilic attack would be directed towards the alkyl group, since carbon is more electropositive than nitrogen or oxygen. It is suggested that the de-ethylation of diethyltin may proceed as follows:

$$(C_2H_5)_2Sn^{2+} \xrightarrow{+OH^-} (C_2H_5)(OH)Sn^{2+} + C_2H_5^- \xrightarrow{+2H^+} C_2H_5Sn^{3+} + C_2H_6 + H_2O$$

Since monoethyltin occurs in the urine and faeces after intraperitoneal injection of diethyltin, but is not excreted in the bile and is not absorbed from the intestine, and yet diethyltin is excreted in the bile, it can be deduced that de-ethylation of diethyltin occurs both in the tissues and in the gut, the intestinal flora probably being responsible for de-ethylation in the gut. The experiment quoted in Table 6 supports the view that the dealkylation can occur in the gut contents. Preliminary attempts to demonstrate de-ethylation of diethyltin by liver homogenates were inconclusive.

Diethyltin dichloride is extensively excreted in the bile by the rat, but ethyltin trichloride is barely excreted at all by this route. Other work in this Laboratory has shown that, for extensive biliary excretion in the rat, a compound must be highly polar and possess a minimum molecular weight in the region of 325 ± 50 (Millburn, Smith & Williams, 1967). These two alkyltins are polar and their molecular weights (248 for Et_2SnCl_2; 245·5 for $EtSnCl_3$) are similar but less than the minimum molecular weight proposed for extensive biliary excretion (see Williams, Smith & Millburn, 1965). Neither of these two compounds could be expected to be excreted in the bile in quantity on the hypotheses of Millburn *et al.* (1967). However, diethyltin dichloride could undergo hydrolysis at the pH of bile (8·4 in the rat) to form tetrameric molecules. These compounds have been described and isolated by Alleston, Davies, Hancock & White (1963) and formulated as $[Et_4SnCl_2O]_2$ and $[Et_4Sn_2Cl(OH)O]_2$. These have molecular weights of 827·5 and 845 respectively. Diethyltin dichloride could therefore be excreted in the bile as these high-molecular-weight hydrolysis products. The final hydrolysis product is the polymeric diethyltin oxide, $[Et_2SnO]_n$, which can be reconverted into diethyltin^{2+} in an acid medium. It was observed that when diethyltin dichloride was injected intraperitoneally into biliary-cannulated rats impaired bile flow occurred in several animals after about 4hr. This could be due to the formation of these polymers, although other explanations such as a toxic effect on the liver are feasible.

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