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The Biochemistry of Organotin Compounds

THE CONVERSION OF TETRAETHYLTIN INTO TRIETHYLTIN IN MAMMALS

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In their study on the toxicity of organotin compounds Stoner, Barnes & Duff (1955) noted that when tetraethyltin was given to rabbits the symptoms after an initial latent period closely resembled those of triethyltin poisoning. They suggested that tetraethyltin was converted into triethyltin. A similar description of the symptoms of tetraethyltin poisoning in rabbits, particularly muscular weakness and lowered body temperature, has been made by Lecoq (1954) and a whole series of these tetra-alkyltin compounds has been studied by Meynier (1956). Previous work (Cremer, 1957) has shown that there is a close correlation between the amount of triethyltin given to rats, the concentration attained in the brain and the degree of inhibition of the metabolism *in vitro* of brain slices prepared from treated rats. An analytical procedure for determining triethyltin in biological media was also described. By similar techniques to those used in that study it has now been possible to show that tetraethyltin is converted into triethyltin by rats and rabbits. Studies on the conversion mechanism *in vitro* have been made and the liver has been found to be the organ most active in converting tetraethyltin into triethyltin.

MATERIALS AND METHODS

The animals used were albino rats of 200–230 g. body wt., maintained on M.R.C. diet no. 41 B (Bruce & Parkes, 1956), and cross-bred rabbits fed on M.R.C. diet no. 18 (Bruce, 1947).

Brain slices were prepared and used as previously described (Cremer, 1957).

Preparation and fractionation of liver homogenates. Fractionation of a 10% (w/v) liver suspension in ice-cold 0·15 M-NaCl–0·015 M-$MgSO_4$–0·008 M-nicotinamide (Davison, 1955) was essentially by the method of Schneider (1948). Liver was homogenized in a Potter–Elvehjem type of homogenizer with a Perspex pestle, as described by Aldridge (1957). Cells and nuclei were removed by centrifuging at 600 *g* for 10 min., mitochondria by a further 20 min. at 5000 *g* and microsomes by a further 120 min. at 20 000 *g*. Each sediment was resuspended without washing to half the original volume of the whole homogenate. For routine experiments the supernatant from a 10% (w/v) liver homogenate in NaCl–$MgSO_4$–nicotinamide medium, centrifuged for 10 min. at 5000 *g*, was used.

Protein was estimated by the biuret method of Robinson & Hogden (1940) as modified by Aldridge (1957), with bovine-serum albumin as the standard.

Purification of tetraethyltin. Tetraethyltin was obtained from Dr G. J. M. van der Kerk. Triethyltin impurity in the sample was removed by several extractions of a solution of tetraethyltin in $CHCl_3$ with N-H_2SO_4 in subdued light, followed by washing with water, borate–ethylenediaminetetraacetic acid (EDTA) buffer ($Na_2B_4O_7$,$10H_2O$, 19 g.; boric acid, 12 g.; EDTA, 4 g.; in 1 l. of water. pH 8·4) (Cremer, 1957), conductivity water and evaporating the $CHCl_3$ *in vacuo*. The purified sample was stored in the dark at 0°. A stock ethanolic solution of tetraethyltin (40 mg./ml.) was prepared and stored in the dark at 0°. This solution was used for intravenous injection. For addition to Warburg flasks small samples of the ethanolic solution were pipetted rapidly into larger volumes of the saline media and shaken vigorously, the suspension being prepared just before use.

Estimation of triethyltin. Triethyltin was estimated in biological material by the dithizone method previously described (Cremer, 1957). The percentage recoveries for the various tissues given in that work have been used to calculate the triethyltin/g. of tissue wet wt. Purified tetraethyltin does not form a complex with dithizone (Aldridge & Cremer, 1957). For experiments *in vitro* triethyltin was esimated in the total flask contents and expressed as μg. found/g. wet wt. for liver slices or μg./100 mg. of protein in cell-fractionation experiments.

Distribution coefficients for organotin compounds. Samples of blood (1 ml.) and liver (1 g.) from rats given 20 mg. of tetraethyltin/kg. intravenously were homogenized in 15 ml. of water. Tartaric acid (10%, w/v, 5 ml.) and perchloric acid (36%, v/v, 5 ml.) were added with efficient mixing and, after centrifuging, a sample (20 ml.) of the supernatant was neutralized with 5N-NaOH (approx. 6 ml.). Borate–EDTA buffer (2 ml.) was added and the mixture treated according to the scheme shown in Fig. 1. The first step in this scheme allowed the organotin compounds present to distribute between the chloroform phase *A* and the aqueous phase *B*. It has previously been shown (Aldridge & Cremer, 1957) that triethyltin distributes in favour of $CHCl_3$, whereas diethyltin remains entirely in the aqueous phase. Triethyltin in the chloroform layer *A* was determined by adding 3 ml. of borate–EDTA buffer, followed by 1 ml. of dithizone reagent [0·004% (w/v) of dithiocarbazone in $CHCl_3$] in subdued light. After mixing for 3 min. the borate buffer was removed by suction and the $CHCl_3$ layer read against a dithizone control in 2 cm. cells at 610 mμ in a Unicam SP. 600 spectrophotometer. Two drops of acetic acid (AnalaR) were then added to each cell and the readings repeated. The organotin compounds do not complex with dithizone under acid conditions so that the difference in the readings gave the amount of dithizone used. The organotin equivalent of this dithizone was obtained from calibration curves prepared by adding 1 ml. of dithizone reagent to 10 ml. of $CHCl_3$ containing known amounts of organotin compounds. The aqueous phase *B* would contain diethyltin and the remaining triethyltin not extracted by $CHCl_3$ if both these compounds were present in the original sample. These organotin compounds formed complexes with the dithizone reagent and the complexes were extracted with $CHCl_3$ (Fig. 1, *C*). The amount of the complexed dithizone was determined as described above. When both di- and tri-ethyltin were present the absorption at 610 mμ was the sum of each organotin–dithizone complex.

Conversion of tetraethyltin by liver slices. The following general procedure was used. A suspension of tetraethyltin (3 ml. of 400 μg./ml.) in Krebs–Ringer phosphate solution (Umbreit, Burris & Stauffer, 1951) with 0·01 M-glucose was added to each flask and liver slices from 200 to 300 mg. total wet wt. The centre well contained 0·2 ml. of 20% (w/v) KOH for CO_2 absorption. After gassing for 3 min. with O_2 the flasks were incubated at 37° and at desired time intervals removed from the bath, the slices homogenized in 5 ml. of water and then added to the remaining flask contents for triethyltin analysis.

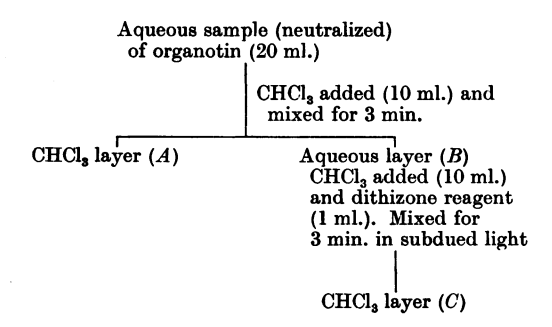

Fig. 1. Scheme of analysis of the distribution of organotin compounds.

Conversion of tetraethyltin by liver-cell fractions. The following general procedure was used; any deviations are described in the text or in the legends to particular experiments. Each flask contained 2 ml. of liver-cell fraction preparation in NaCl–$MgSO_4$–nicotinamide medium, tetraethyltin 0·0012 M, diphosphopyridine nucleotide (DPN) 0·00018 M, in a total volume of 3 ml. The centre well contained 0·2 ml. of 20% (w/v) KOH for CO_2 absorption. Incubation was at 37°. Flasks were gassed for 3 min. with O_2 and at desired time intervals 5 ml. of 10% (w/v) tartaric acid was added and triethyltin estimated in the total flask contents.

Special chemicals. DPN was prepared from baker's yeast by the method of Kornberg & Pricer (1953), as described by Aldridge & Cremer (1955).

Triphosphopyridine nucleotide (TPN) was prepared from cow liver as described by Threlfall (1957).

RESULTS

Conversion of tetraethyltin in vivo

Male rats were given intravenous (tail vein) injections of tetraethyltin (20 mg./kg. body wt.) and killed at intervals after the injection. Liver, kidney, brain and whole-blood samples were examined for triethyltin. Brain slices were prepared from the same rats and their O_2 consumption, pyruvate and lactate levels determined *in vitro*. The results given in Table 1 show a definite conversion of tetraethyltin into triethyltin. The distribution of the triethyltin formed closely resembled the pattern of distribution obtained in rats given triethyltin itself (Cremer, 1957). The metabolism of the brain slices was altered in a similar manner to that of rats treated with triethyltin and again there appeared to be a close correlation between the amount of triethyltin found in the brain and the degree of alteration in its metabolism *in vitro*. This can be seen more clearly in Table 2, where the concentration of triethyltin found in the brain has been expressed as the molar concentration in the tissue fluid as described previously (Cremer, 1957). The values of Q_{O_2} and lactic acid:pyruvic acid ratios have been compared with those of brain slices to which triethyltin was added *in vitro*.

A large quantity of triethyltin was found in the liver, with smaller amounts in the kidney, brain and whole blood in a rabbit killed in a prostrate condition 2 hr. after an intravenous injection of tetraethyltin (25 mg./kg., Table 1).

Effect of SKF 525-A on conversion in vivo. Axelrod, Reichenthal & Brodie (1954) first demonstrated that the compound 2-diethylaminoethyldiphenylpropylacetate hydrochloride (SKF 525-A) potentiated the action of barbiturates by retarding their rate of metabolic transformation. Since then several other workers have shown that SKF 525-A

prevents or retards the metabolic breakdown of a variety of foreign substances given to animals (Davison, 1955; Magee, 1956). Four rats were given SKF 525-A intraperitoneally (100 mg./kg.) followed after 40 min. by tetraethyltin intravenously (20 mg./kg.) and killed 60 min. after the latter injection. The level of triethyltin in blood and liver and the activity of brain slices *in vitro* were determined (Table 1). SKF 525-A slightly reduced the conversion of tetraethyltin, but did not prevent it. However, a more striking effect of SKF 525-A on the conversion of tetraethyltin was found in systems *in vitro* to be described later.

Tissue-water content after tetraethyltin in vivo. Magee, Stoner & Barnes (1957) determined the water content of the brain and spinal cord of rats given triethyltin hydroxide and showed there was a marked increase. Two 200 g. male rats given an intravenous injection of tetraethyltin (20 mg./kg.) were killed 55 and 76 hr. later. The brains and spinal cords were dissected out, samples taken for histological examination and the water content was determined on the remainder by drying to constant weight at 105°. The mean values found were 80·78 and 77·0% for the water content of brain and spinal cord respectively compared with 77·2 ± 0·39% S.D. and 69 ± 0·39% S.D. for control rats of this body weight (Dr H. B. Stoner, personal communication). The increased water content in rats treated with tetraethyltin was in very close agreement with the results of Magee *et al.* (1957) from acute experiments with triethyltin sulphate. The histological findings confirmed the presence of an interstitial oedema of the white matter.

Proof that triethyltin is formed from tetraethyltin

The distribution coefficient between $CHCl_3$ and water of diethyltin and triethyltin alone and of mixtures of known concentrations have been compared with those of a substance or substances combining with dithizone from blood and liver samples from rats given tetraethyltin. The results

Table 1. *Conversion of tetraethyltin* in vivo

For brain-cortex metabolism *in vitro* each flask contained 3 ml. of Krebs–Ringer phosphate soln. with 0·011 M-glucose and 68 mg. of tissue slice (average wet wt.). The centre well contained 0·2 ml. of 20% (w/v) KOH for CO_2 absorption. After incubation at 37° for 75 min. slices were removed for dry-weight determination and 3 ml. of 18% (w/v) trichloroacetic acid was added to the remaining flask contents. After centrifuging, lactic and pyruvic acid were determined in samples of the supernatant. Q_{O_2} is expressed as the μl. of O_2/mg. dry wt./hr.; lactic and pyruvic acid are given as the ratio total wt. (μg.) of lactic acid found/flask: total wt. (μg.) of pyruvic acid found/flask. Triethyltin is expressed as μg./g. of tissue wet wt. Mean values are given with the standard error of the mean (S.E.M.) for each value.

Species	Time after injection of tetraethyltin (min.)	No. of animals	Brain cortex slices		Triethyltin (μg./g.)			
			Q_{O_2}	Lactic acid/ pyruvic acid	Blood	Liver	Kidney	Brain
Rat	Normal	3	12·4 ± 0·87	16·2 ± 1·11	—	—	—	—
	30	4	13·5 ± 1·09	17·1 ± 0·04	3·94 ± 1·18	6·3 ± 1·19	1·99 ± 0·05	0·26 ± 0·01
	60	4	11·03 ± 0·05	38·4 ± 4·7	31·9 ± 3·45	18·1 ± 1·06	5·6 ± 1·4	0·66 ± 0·01
	60*	4	11·2 ± 1·03	41·2 ± 10·1	27·0 ± 4·8	12·5 ± 4·8	—	—
	120	4	8·52 ± 1·14	72·4 ± 13·9	34·3 ± 4·71	18·0 ± 2·27	6·9 ± 0·82	2·14 ± 0·61
Rabbit	90	1	—	—	1·3	40·8	16·1	4·7

* Rats were given SKF 525-A; see text.

Table 2. *Comparison of the activity of brain slices with triethyltin added* in vitro *and slices from rats given tetraethyltin*

For details of the calculation of the molar concentration of triethyltin in the brain see text and Cremer (1957).

Concn. of triethyltin in tissue fluid (M)			
Added *in vitro*	Found *in vivo* after intravenous injection of tetraethyltin	Q_{O_2}	Lactic acid/ pyruvic acid
—	—	12·4	16·2
$5·3 \times 10^{-6}$	—	13·5	45·0
$1·58 \times 10^{-5}$	—	9·0	106·0
—	$3·2 \times 10^{-6}$	11·03	38·0
—	$1·05 \times 10^{-5}$	8·52	72·0
Tetraethyltin $1·5 \times 10^{-4}$	—	12·9	20·0

(Table 3) show that the distribution coefficients of the tissue samples were very similar to that of triethyltin. The procedure is not satisfactory for quantitative measurements of diethyltin, particularly small amounts, but if more than 10 μg. was present in tissues the ratio R would be lower than that of triethyltin. It would seem therefore that the substance in the blood and liver samples which formed a complex with dithizone was triethyltin.

As a further proof of the formation of triethyltin from tetraethyltin *in vivo* the absorption of the dithizone complex formed in $CHCl_3$ extracts of blood samples from rats injected with tetraethyltin were examined. Aldridge & Cremer (1956) have previously shown that the triethyltin–dithizone complex at pH 8·75 is converted into diethyltin–dithizone complex by exposure to strong light, the absorption maximum changing from 440 to 485 mμ. Table 4 shows the absorption maxima for triethyltin–dithizone before and after exposure to sunlight for 5 min. and those obtained after identical treatment of a blood sample from a rat injected with tetraethyltin. The absorption curves for the blood sample both before and after exposure to intense light were identical in shape and height with those of the control triethyltin.

Conversion of tetraethyltin in vitro

Tissue slices. Systems were studied *in vitro* in an attempt to locate the site of conversion of tetraethyltin. Tissue slices of liver, kidney, brain and small intestine and samples of whole blood were examined for their ability to convert tetraethyltin into triethyltin. Liver slices were the most active, although a definite but small amount of triethyltin was found in kidney slices. The other tissues were completely inactive. Since *in vivo* the highest concentration of triethyltin in treated rats is found in erythrocytes it was of interest that no triethyltin was found in whole blood incubated with tetraethyltin. Fig. 2 shows the rate of formation of triethyltin by rat- and rabbit-liver slices incubated with tetraethyltin. For rat-liver slices the rate is linear up to 90 min. and there is a direct relationship between the wet weight of liver tissue and the amount of triethyltin found.

Table 3. *Distribution of organotin compounds between chloroform and water*

Blood (1 ml.) and liver (1 g.) samples were from rats injected with tetraethyltin, as used in Table 1. The layers A and B refer to the scheme in Fig. 1. Details of their composition are given in Methods.

Organotin compounds		Organotin–dithizone complexes (optical density at 610 mμ)		
Triethyltin (μg.)	Diethyltin (μg.)	$CHCl_3$ layer (A)	Aqueous layer (B)	$R = A/B$
8·65	—	0·182	0·048	3·8
17·3	—	0·391	0·106	3·7
26·0	—	0·545	0·156	3·5
—	8·6	0	0·066	0
—	17·2	0	0·169	0
—	25·8	0	0·262	0
8·65	25·8	0·186	0·267	0·7
17·3	17·2	0·39	0·219	1·78
26·0	8·6	0·546	0·222	2·45
Tissue samples from rats given tetraethyltin				
Blood		0·43	0·11	3·9
		0·605	0·194	3·1
		0·706	0·145	4·86
Liver		0·153	0·028	5·46
		0·276	0·104	2·7
		0·30	0·085	3·53

Table 4. *Absorption maxima for triethyltin–dithizone complexes*

The blood sample (2 ml.) was from a rat killed 60 min. after an intravenous injection of tetraethyltin (20 mg./kg.). Values are given as optical-density readings at the maxima of two wavelengths.

	Before intense light		After intense light	
	440 mμ	485 mμ	440 mμ	485 mμ
Triethyltin (43 μg.)	0·595	0·29	0·33	0·62
Blood from rat given tetraethyltin	0·60	0·29	0·333	0·62

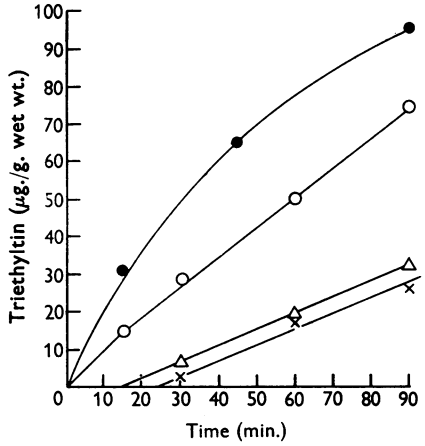

Fig. 2. Conversion of tetraethyltin by liver slices. The results are plotted as μg. of triethyltin found/g. of liver slices wet wt. Each flask contained 3 ml. of tetraethyltin suspension (400 μg./ml. in Krebs–Ringer phosphate solution) with 0·011 M-glucose and liver slices (250 mg. average wet wt.). Incubation was at 37° in O_2. ●, Normal rabbit-liver slices; ○, normal rat-liver slices; △, liver slices from rat injected with SKF 525-A (100 mg./kg.); ×, rat-liver slices with SKF 525-A (0·17 mM) added *in vitro*.

SKF 525-A added *in vitro* at 0·17 mM to liver slices was found to slow the conversion of tetraethyltin (Fig. 2). Cooper, Axelrod & Brodie (1954) have shown that the rate of metabolism of hexobarbital by liver slices from rats treated with SKF 525-A is markedly lower than that of slices from an untreated animal. Very similar results were obtained in an experiment in which a rat was given an intraperitoneal injection of SKF 525-A (100 mg./kg.) and killed 40 min. later, and from which liver slices were prepared and the rate of conversion of tetraethyltin compared with that in liver slices from an untreated rat (Fig. 2).

In studies on the metabolism of foreign compounds several workers have noted differences in the activity of liver from male and female rats (Quinn, Axelrod & Brodie, 1954; Davison, 1955; Fenwick, Barron & Watson, 1957; Du Bois, Thush & Murphy, 1957). Female rat-liver slices converted tetraethyltin at a slower rate than male rat-liver slices (Table 5).

Conversion by liver-homogenate preparations

The fractions obtained by differential centrifuging techniques from liver homogenates prepared in NaCl–$MgSO_4$–nicotinamide medium were tested for their ability to convert tetraethyltin (Table 6). The amount of triethyltin produced by each fraction has been expressed both as total μg./flask and as μg./100 mg. of protein to enable a strict comparison of relative activities to be made. The microsome plus clear supernatant fraction was the most active and was double the sum of the two fractions alone.

Experiments were carried out to determine the requirements of the liver microsome plus supernatant fraction for maximal conversion activity.

Table 5. *Comparison of tetraethyltin conversion by liver slices of male and female rats*

Details were as described in Methods. Male and female albino rats of 200 g. were used. Values are averages for two rats in each group.

	Triethyltin found (μg./g. wet wt.)			
	15 min.	30 min.	60 min.	90 min.
Male	19·7	29·6	50·0	76·6
Female	1·3	4·2	17·3	28·5

Table 6. *Distribution of the tetraethyltin-converting system in rat-liver cells*

Each precipitate was made up to half the volume of the original 10% (w/v) homogenate in either 0·15 M-NaCl–0·015 M-$MgSO_4$–0·008 M-nicotinamide or clear supernatant. Each flask contained 2 ml. of liver suspension, DPN 0·00025 M and tetraethyltin 0·0011 M (1 mg./flask) final concn. in a volume of 3 ml. Flasks were incubated at 37° in O_2 for 90 min., and determinations of triethyltin were then made.

	Triethyltin found	
Liver fraction	μg./flask	μg./100 mg. of protein
Whole homogenate	9·0	34·0
Cell debris + nuclei	0	0
Mitochondria	1·3	23·0
Microsomes	1·34	28·4
Supernatant	6·4	47·7
Mitochondria + supernatant	10·6	64·0
Microsomes + supernatant	13·8	81·0

Table 7. *Effect of addition of fortifying constituents on the conversion of tetraethyltin by rat-liver preparations*

Samples (2 ml.) of the supernatant from a 10% (w/v) liver homogenate in 0·15 M-NaCl centrifuged at 5000 *g* for 20 min. at 0° were added to each flask and tetraethyltin 0·001 M final concn. Magnesium sulphate 0·015 M, nicotinamide 0·008 M, DPN 0·00018 M and TPN 0·00019 M final concn. were added where indicated in a total volume of 3 ml. Incubation was at 37° in O_2 for 90 min., and determinations of triethyltin were then made.

Fortification	Triethyltin found (μg./100 mg. of protein)
None	9·45
$MgSO_4$	11·7
Nicotinamide	48·0
Nicotinamide + $MgSO_4$	38·5
Nicotinamide + DPN	64·0
Nicotinamide + DPN + $MgSO_4$	81·5
Nicotinamide + TPN	136·0
Nicotinamide + TPN + $MgSO_4$	161·0

Results are given in Table 7, and show the effects of adding various combinations of $MgSO_4$, nicotinamide, DPN and TPN. The highest results were obtained with the combination of $MgSO_4$, nicotinamide and TPN.

The rate of conversion of tetraethyltin by rabbit- and rat-liver microsomes plus supernatant preparations is shown in Fig. 3. In rat liver the conversion was most rapid during the first 20 min. of the incubation. The overall activity of rabbit liver was higher than that of the rat.

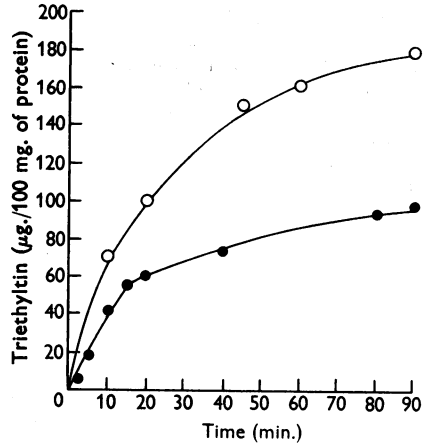

Fig. 3. Rate of conversion of tetraethyltin by rabbit- (○) and rat- (●) liver microsome plus supernatant fraction. Samples (2 ml.) of the supernatant from a 10% (w/v) liver homogenate prepared in 0·15 M-NaCl–0·015 M-$MgSO_4$–0·008 M-nicotinamide medium centrifuged for 20 min. at 5000 *g* were added to each flask with tetraethyltin 0·0011 M and DPN 0·00018 M final concn. in a volume of 3 ml. Incubation was at 37° in O_2. Flasks were removed at required time intervals for determinations of triethyltin.

Table 8. *Inhibition of rat-liver microsome plus supernatant fraction conversion of tetraethyltin*

A volume (2 ml.) of the supernatant from a 10% (w/v) liver homogenate in 0·15 M-NaCl–0·015 M-$MgSO_4$–0·008 M-nicotinamide centrifuged at 5000 *g* for 20 min. at 0° was added to each flask with tetraethyltin 0·0011 M and DPN 0·00018 M final concn. and SKF 525-A, where indicated, in a volume of 3 ml. Incubation was at 37° in O_2, except where stated otherwise.

Treatment	Triethyltin found (μg./100 mg. of protein) 15 min.	90 min.
Control	55·5	117
SKF 525-A (0·51 mM)	0	8
SKF 525-A (0·17 mM)	5·8	16
Incubated in N_2	15	15
Liver fraction in boiling-water bath for 5 min.	0	0

The microsome plus supernatant preparation of rat liver was sensitive to SKF 525-A, 0·17 mM causing over 80% inhibition of the conversion of tetraethyltin (Table 8). Incubation in an atmosphere of N_2 almost completely inhibited the conversion, as did placing the liver preparation in a boiling-water bath for 5 min. just before use (Table 8).

DISCUSSION

The accumulated evidence presented in the results, showing inhibition of the metabolism of brain slices, increase in water content of the brain and spinal cord and the identification of triethyltin in tissues of animals given tetraethyltin, leaves little doubt that tetraethyltin is converted into triethyltin *in vivo* by rats and rabbits. Triethyltin has been shown to be the main conversion product but it has not been possible to decide whether or not any further breakdown products such as di- and monoethyltin are formed in small amounts.

Since the symptoms of poisoning from a dose of tetraethyltin develop only after an initial latent period, and the increased inhibition of brain-slice metabolism can be closely correlated with increased amounts of triethyltin found in the brain, it seems reasonable to suggest that the toxic action of tetraethyltin is due solely to the triethyltin formed from it by the liver. Tetraethyltin added *in vitro* at a concentration of 0·15 mM had no effect on metabolism of brain slices (Table 2).

The conversion of tetraethyltin *in vitro* has many points in common with the metabolism of drugs by liver preparations (Cooper & Brodie, 1954; Davison, 1955; Fenwick *et al.* 1957). Liver slices have a relatively high conversion activity without requiring additional cofactors, whereas liver-homogenate preparations have a low activity unless fortified. In the initial fractionation experiments carried out both in the saline medium and in 0·25 M-sucrose the activity of the microsome plus clear supernatant fraction was equal only to the sum of the activity of the two fractions alone. When the triethyltin found was expressed as μg./100 mg. of protein the clear supernatant was the most active fraction. In later experiments (Table 6), however, when the microsome and clear supernatant fractions were combined they showed a stimulated activity. No explanation can be given for the difference in the results, but since the values quoted were from later experiments there may possibly have been improvements in the technique of preparing the fractions. The mitochondrial fraction always contained some conversion activity, even after it had been washed with 0·25 M-sucrose. Brodie (1956) has recently reviewed very fully the work on drug metabolism by liver preparations and poses the question 'does

each type of metabolic pathway involve the same enzyme system?'. Some differences have been found, and yet, considering the wide diversity in the chemical composition of the drugs metabolized, the requirements and behaviour of the liver microsome plus supernatant fraction are remarkably similar. The conversion described in this paper also conforms to Brodie's concept that the metabolism of foreign compounds by the liver makes the products less lipid-soluble than the original compound. The way in which SKF 525-A acts is not known, but the concentration at which it inhibits the conversion of tetraethyltin into triethyltin is very similar to that at which it inhibits metabolism of hexabarbitone, amidopyrine and pethidine (Brodie, 1956).

A correlation between the relative toxicity of tetraethyltin *in vivo* to male and female rats and the difference in their rates of converting tetraethyltin into triethyltin is complicated by the fact that the two sexes differ in their sensitivity towards triethyltin (Barnes & Stoner, 1958). Thus although female rats convert tetraethyltin more slowly, they are nearly twice as sensitive to triethyltin as male rats.

The present results strongly suggest that the conversion of tetraethyltin is enzymic. The chemistry of the conversion has not been proved but it would appear to involve an oxidative mechanism. An attempt to convert tetraethyltin chemically by using the model system described by Brodie, Axelrod, Shore & Udenfriend (1954), in which hydroxyl groups are produced by a reaction product of hydrogen peroxide and ascorbic acid, seemed to destroy triethyltin too rapidly for its detection, whereas triethyltin is relatively stable both *in vivo* and in biological material *in vitro*. However, triethyltin can be detected in a sample of tetraethyltin after storage in light and air for several weeks and some conversion occurs within a few hours when a dilute chloroform solution of tetraethyltin is exposed to intense light.

The finding that tetraethyltin can be converted into triethyltin *in vivo* may have some bearing on the toxic mechanism of tetraethyl lead, a compound which has interested people for over 20 years owing to its widespread use in petroleum. Both Buck & Kumro (1930) and Machle (1935) have suggested that the action of tetraethyl lead may be due to more water-soluble products of decomposition, particularly triethyl lead salts, but no results have been published to substantiate this idea.

SUMMARY

1. Triethyltin has been detected in the tissues of rabbits and rats given tetraethyltin by intravenous injection. The chemical behaviour of the compound found in the tissues is compared with a pure sample of triethyltin and the two are shown to be almost identical.

2. Brain slices prepared from rats given tetraethyltin show a lowered metabolism *in vitro* similar to that of brain slices from rats given triethyltin. Tetraethyltin added to brain slices *in vitro* is without effect.

3. Examination of various tissue slices for their ability to convert tetraethyltin into triethyltin shows the liver to be the most active organ.

4. The converting system is in the microsomes plus soluble material of liver cells. It requires nicotinamide, magnesium sulphate and triphosphopyridine nucleotide for maximal activity and is inhibited by lack of oxygen and by SKF 525-A (2-diethylaminoethyldiphenylpropylacetate hydrochloride).

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South African Pilchard Oil

7. THE ISOLATION AND STRUCTURE OF AN OCTADECATETRAENOIC ACID FROM SOUTH AFRICAN PILCHARD OIL*

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Toyama & Tsuchiya (1935) isolated an octadecatetraenoic acid (moroctic acid) from sardine oil and assigned to it a 4:8:12:15-tetraene structure. Paschke & Wheeler (1954) reported the presence of a tetraethenoid octadecanoic acid in the freshwater alga *Chlorella pyrenoidosa*, but were unable to isolate it. By analogy with other acids isolated from this source, and from spectroscopic evidence, they considered a 6:9:12:15-tetraene structure as most probable. Recently, the isolation of octadeca-6:9:12:15-tetraenoic acid from herring oil was reported by Klenk & Brockerhoff (1957). The evidence for the presence of such an acid in pilchard oil was also obtained in this Laboratory (see Sutton, 1957, quoting unpublished work by Whitcutt).

The isolation of an octadecatetraenoic acid from South African pilchard oil (*Sardina ocellata* Jenyns) is now reported. The methods of separation and identification were the same as those described in previous papers of this series (see Silk & Hahn, 1954*b*; Whitcutt & Sutton, 1956). The acid is shown to be the all-*cis*-*n*-octadeca-6:9:12:15-tetraenoic.

EXPERIMENTAL AND RESULTS

Melting points are uncorrected. General methods were described in previous papers of this series.

Infrared and ultraviolet spectra were determined on Perkin–Elmer model 21 and Unicam SP. 500 spectrophotometers respectively.

* Part 6: Whitcutt, 1957

Isolation of the octadecatetraenoic acid

Molecular distillation of the unsaturated esters. The starting material for the isolation of the octadecatetraenoic acid was distillate 1, Table 1 of part 6 of this series (Whitcutt, 1957), which was prepared by lithium salt–acetone and urea-complex procedures for segregation of the acids,

Table 1. *Molecular distillation of distillate* 1, *Table* 1, *part* 6 *of this series* (*Whitcutt*, 1957)

Fraction no.	Distillation temp.	Wt. (g.)
1	60–70°	39·9
2	70	9·7
3	70	7·5
4	70	5·9
R (Residue)	—	69·0

Table 2. *Molecular distillation of fraction* 1, *Table* 1

Fraction no.	Distillation temp.	Wt. (g.)
1*a*	52°	1·85
1*b*	56	3·35
1*c*	56	6·20
1*d*	60	4·94
1*e*	60	3·71
1*f*	60	3·05
1R (Residue)	—	13·20

Table 3. *Molecular distillation of fraction* 1*R*, *Table* 2

Fraction no.	Distillation temp.	Wt. (g.)
1RD	64°	6·25
1RR (Residue)	—	2·93