The Interaction of Triethyltin with Components of Animal Tissues

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1. The distribution of triethyl[^{113}Sn]tin chloride in the rat, guinea pig and hamster is not uniform, the highest concentrations being in rat blood and the liver of all three species. 2. Subcellular fractionation of rat liver, brain and kidney shows that triethyltin binds to all fractions to different extents. In the liver of the rat and guinea pig the supernatant fraction contains the largest amount and the highest specific concentration; this triethyltin is bound to a non-diffusible component. 3. Rat haemoglobin is responsible for the binding of triethyltin in rat blood (2 moles of triethyltin/mole of haemoglobin). Haemoglobins from other species have much less affinity for triethyltin. 4. A variety of other proteins do not bind triethyltin.

Triethyltin possesses some interesting biological properties. It is toxic to mammals, the lethal dose to rats being 10 mg./kg. body wt. (Stoner, Barnes & Duff, 1955). The only pathological change is an oedema of the white matter of brain and spinal cord (Magee, Stoner & Barnes, 1957; Streicher, 1962; Katzman, Aleu & Wilson, 1963). Triethyltin inhibits oxidative phosphorylation *in vitro* at concentrations of 0.1–1.0 μM (Aldridge, 1958; Aldridge & Threlfall, 1961; Moore & Brody, 1961; Sone & Hagihara, 1964).

Neither the toxicity, nor the induction of cerebral oedema nor the inhibition of oxidative phosphorylation have been explained in chemical terms. Trialkyltins are relatively inert in non-biological aqueous systems and there is evidence that they have little affinity for the SH groups of reduced glutathione and 2,3-dimercaptopropan-1-ol (Aldridge & Cremer, 1955). Affinity for phospholipids has been demonstrated (Aldridge & Street, 1964) and inhibition of [^{32}P]phosphate incorporation into rat brain phospholipids was observed after injection of triethyltin (Stoner & Threlfall, 1958). However, this inhibition of incorporation was shown not to be a metabolic consequence of a direct interaction of the triethyltin with phospholipids but a secondary effect arising from the fall in body temperature of rats poisoned with triethyltin (Rose & Aldridge, 1966).

In order to try to understand the toxic action of these compounds, information is required about molecular groupings that have affinity for trialkyltin. Such information may be obtained from studies of the basic chemistry of trialkyltins, i.e. their reactivity with small molecules. In addition,

if a reaction of trialkyltins with pure macromolecules could be studied, the information so derived might have relevance (in a molecular sense) to the inhibition of oxidative phosphorylation and eventually to the disturbances produced in the whole animal. Previous work (Aldridge, 1958) indicates that the molecular groupings (presumably on a macromolecule) involved in the inhibition of oxidative phosphorylation must have an association constant for triethyltin of the order of 10^6 M^{-1} . The aim of the experiments with triethyl[^{113}Sn]tin chloride described in the present paper was to answer two questions: (1) does triethyltin have such affinity for many macromolecules, and (2) are there any macromolecules suitable for further study as model systems?

Distribution studies have been carried out by Cremer (1957, 1958) and Brody & Moore (1962). The stability *in vivo* of the triethyltin moiety demonstrated by them has allowed us to extend their studies by using triethyl[^{113}Sn]tin.

MATERIALS AND METHODS

Special chemicals

Triethyltin chloride labelled with ^{113}Sn was purchased from The Radiochemical Centre, Amersham, Bucks. The specific activity was 0.15 mc/m-mole. This was dissolved in ethanol to give a stock solution of 200 mg./ml (approx. 0.8 M). The following proteins were obtained from the sources stated: egg phosphitin (from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.); clupein sulphate and salmine sulphate (from British Drug Houses Ltd., Poole, Dorset); cytochrome c (from Sigma Chemical Co., St Louis, Mo., U.S.A.); crystalline bovine plasma albumin (from Armour Pharmaceutical Co. Ltd., Eastbourne,

Sussex); chymotrypsin (from Armour Laboratories, Armour and Co. Ltd., London, E.C. 1); horse myoglobin and crystalline bovine pancreatic ribonuclease (from Seravac Laboratories, Maidenhead, Berks.).

Animals

Male albino rats of the Porton strain (200 ± 10 g. body wt.) were used. Guinea pigs were obtained from Allington Farm, Porton Down, Wilts.; golden hamsters were obtained from Stewart's, Ashton, Chester.

Determination of the distribution of triethyltin in whole animals

Triethyl[^{113}Sn]tin chloride in ethanol (200 mg./ml.) was diluted with distilled water and injected via a tail vein into rats and intraperitoneally into hamsters and guinea pigs. In some experiments guinea pigs were anaesthetized with ether and injected intravenously via a saphenous vein. Animals were decapitated at intervals after the injection of triethyltin unless they were to be perfused. In this case, each was anaesthetized with ether, the chest opened and a sample of blood removed from the heart with a heparinized syringe. The gall bladders of guinea pigs and hamsters were emptied by means of a syringe before perfusion. A cannula was then inserted into the aorta through the left ventricle of the heart. Once perfusion with Krebs-Ringer phosphate (Umbreit, Burris & Stauffer, 1964) was started, the abdomen was opened (if it had not been opened for extraction of bile) and clearance of the blood from the organs was assisted by gentle manipulation with the fingers. Perfusion was stopped at various times (3–10 min.). After 3 min. the returning fluid escaping from the heart was usually colourless and inspection of organs such as liver and kidneys indicated satisfactory removal of blood. After decapitation or perfusion, weighed samples of tissues were taken for radioactivity measurements as described below.

Determination of the distribution of triethyltin within tissues

Fractionation of brain. Brain tissue (from two rats) was fractionated and the fractions were washed by the method of Aldridge & Johnson (1959).

Fractionation of liver. Liver tissue (8 g.) was homogenized in 30 ml. of ice-cold 0.3 M-sucrose as described by Aldridge (1957, 1958) and Aldridge & Street (1964) for the preparation of mitochondria. The fractionation and washing procedures were similar to those used for brain. The centrifuging schedules for the following fractions were: nuclear and debris, 850 g for 10 min.; heavy mitochondrial, 5000 g for 15 min.; light mitochondrial, 13 000 g for 15 min.; microsomal, 40 000 g for 60 min.

Fractionation of kidney. Kidney tissue (8 g. from three rats) was fractionated as described for liver, with a homogenization medium of 0.3 M-sucrose containing 1.0 mM-EDTA. The washing procedure was identical with that described for brain (Aldridge & Johnson, 1959).

Rat liver nuclei. Perfused liver (7 g.) from a rat starved for 24 hr. was homogenized in 17 ml. of a solution containing 0.3 M-sucrose, 3 mM-MgCl₂ and 0.1 M-tris-HCl buffer, pH 7.4. The homogenizer used had a difference in diameter between pestle and tube of 0.02 in. and the pestle rotated at 1900 rev./min. (Aldridge, Emery & Street, 1960). This homogenate was mixed with 140 ml. of 2.1 M-sucrose

containing 3 mM-MgCl₂, and was centrifuged for 2 hr. at 40 000 g in an angle rotor of the MSE Angle 18 centrifuge. The supernatant and debris at the bottom of the tube were sucked off and the smear of nuclei down the side of the tube was suspended in the original solution containing sucrose, MgCl₂ and tris-HCl buffer. After sedimenting at 800 g for 10 min. in a swing-out head, the nuclei were resuspended in the same solution.

Determination of protein in fractions. Protein was determined by the biuret method as modified by Aldridge (1962).

Technique for equilibrium dialysis

The binding of triethyltin to various preparations of macromolecules was investigated by using equilibrium dialysis. The preparations (5 ml.) were dialysed in Visking tubing (Viscose Development Co., London, W.C. 2) against Krebs-Ringer phosphate or suitable buffer (5.0 ml.) with gentle shaking at 5°. Under these conditions free triethyltin equilibrated across the membrane with a half-time of 80 min. To ensure that equilibrium was reached shaking was continued overnight (16 hr.).

Measurement of radioactivity

^{113}Sn is a γ -ray-emitting isotope that decays by the process of K-electron capture to yield a metastable indium isotope, a process with a half-life of 118 days:

This metastable indium isotope then undergoes rapid decay (half-life 1.73 hr.) to a stable indium isotope with the emission of a second γ -ray:

The latter γ -ray (391 keV) was monitored since it represents a larger proportion of the radiation emitted. The concentration of ^{113m}In is therefore being measured and not ^{113}Sn .

In the steady state, defined by the equation:

$$k_1[^{113}\text{Sn}] - k_2[^{113m}\text{In}] = 0$$

the concentration of ^{113m}In can be shown to be approx. 0.06% of the concentration of ^{113}Sn ; the rate at which the system reaches the steady state will be proportional to the rate of the fastest step ($^{113m}\text{In} \rightarrow ^{113}\text{In}$, half-life 1.73 hr.). After all experimental manipulations, therefore, samples containing ^{113}Sn were left for at least 16 hr. (9 half-lives) before being counted to ensure that the above steady state had been reached and the ^{113m}In monitored was a true measure of the amount of ^{113}Sn present.

Counting was carried out with a Packard Autogamma scintillation spectrometer with an overall efficiency of 16%. A sealed standard containing 100 μ moles of triethyl- ^{113}Sn]tin and suitable blanks for background measurements were always counted with every batch of samples, thus eliminating the necessity of correction for decay of the isotope.

RESULTS

Distribution of triethyltin in the rat

The distribution of triethyl[^{113}Sn]tin 24 hr. after intravenous injection of 10 mg./kg. body wt. is not uniform (Table 1). In particular, high concentrations are found in the blood and liver. On centri-

fugation of the blood, most of the triethyltin in blood is found in the erythrocytes (Table 5; cf. Cremer, 1957). Animals were perfused before tissue sampling to remove blood from the organs and to see whether the triethyltin could be washed out. Some decreases in concentration were observed (Table 1), but these can be accounted for in kidney and brain by an increase in the wet weight of the tissue due to swelling on perfusion. The same is probably true for the other tissues examined.

Table 1. *Distribution of triethyltin in the rat and the effect of perfusion*

Rats were killed 24 hr. after intravenous injection of 10 mg. of triethyl[^{113}Sn]tin chloride/kg. body wt. Animals were perfused for 3–5 min. The results are given as means $\pm$ s.e.m. (with the numbers of observations in parentheses). The recovery of injected triethyltin in these fractions is 92%, assuming the following weights for the organs of a 200 g. rat: blood, 14 g.; liver, 6 g.; kidney, 2 g.; brain, 2 g.; muscle, 80 g.

Tissue	Triethyltin ($\mu\text{moles/g. wet wt. of tissue}$)	
	Without perfusion	After perfusion
Liver	141.0 $\pm$ 5.8 (3)	108.0 $\pm$ 6.6 (3)
Kidney	89.2 $\pm$ 2.9 (3)	67.7 $\pm$ 1.7 (3)
Muscle	29.0 $\pm$ 0.1 (3)	20.3 $\pm$ 1.7 (3)
Brain		
Brain stem	24.0 $\pm$ 1.2 (3)	19.5 $\pm$ 0.8 (3)
Cerebellum	31.5 $\pm$ 0.8 (3)	24.5 $\pm$ 0.2 (3)
Cerebrum (half)	29.0 $\pm$ 1.7 (3)	23.3 $\pm$ 0.8 (3)
Cortex	32.7 $\pm$ 1.2 (3)	27.8 $\pm$ 1.7 (3)
Remainder of brain	28.2 $\pm$ 1.2 (3)	22.4 $\pm$ 0.8 (3)
Spinal cord	21.6 $\pm$ 2.5 (3)	15.5 (2)
Fat	51.8 (1)	41.8 $\pm$ 5.8 (3)
Blood	297.0 $\pm$ 11.6 (6)	

Differences in triethyltin content within different areas of the brain are not significant. Concentrations of triethyltin in fractions rich in white matter may be decreased at 24 hr. owing to their increased water content (Magee *et al.* 1957).

After intravenous injection of 10 mg. of triethyltin chloride/kg. body wt., equilibrium between tissues is almost reached at 1 hr. and is 60–70% complete at 10 min. (Table 2). The concentration of triethyltin in most tissues falls only slowly, 40–60% of the injected dose remaining at 5 days, with more rapid loss from blood and fat. In other experiments rats injected with 5 mg. of triethyltin chloride/kg. body wt. were kept in metabolism cages. In 4 days, 50% of the dose was excreted, mostly in the faeces. Triethyltin is probably secreted in the bile of the rat, as it is in the guinea pig and hamster (Table 3).

At the lowest dose used (0.625 mg. of triethyltin chloride/kg. body wt.) the concentrations of triethyltin in the tissues are in the order liver > blood = kidney > brain (Fig. 1). As the dose is increased there is a proportional increase in the concentration in each tissue, and saturation in any tissue is not reached. However, the relationship between dose and concentration in each tissue is different so that there is a change in the concentration in one tissue relative to another, and at the highest dose (5.0 mg./kg. body wt.) blood contains more than the liver (Fig. 1). This suggests that rat liver has a greater affinity for triethyltin than has rat blood.

Distribution of triethyltin in the hamster and guinea pig

The most surprising feature of the distribution of triethyltin in hamster and guinea pig (Table 3) compared with that in the rat is the small amount

Table 2. *Distribution of triethyltin in the rat at various times after dosing*

Rats were injected intravenously with 10 mg. of triethyl[^{113}Sn]tin chloride/kg. body wt. All animals were perfused. A single rat was used at each separate time with the exception of 24 hr. where the values given are those from Table 1.

Tissue	Triethyltin ($\mu\text{moles/g. wet wt. of tissue}$)				
	10 min.	60 min.	4 hr.	24 hr.	5 days
Liver	79.0	101.0	103.0	108.0	63.0
Kidney	42.3	48.5	52.7	67.7	30.3
Muscle	11.2	14.1	16.2	20.3	7.5
Brain					
Brain stem	13.7	19.9	22.0	19.5	8.3
Cerebellum	14.5	15.8	22.4	24.5	10.4
Cerebrum (half)	12.9	19.9	22.4	23.3	10.0
Cortex	11.6	22.0	21.2	27.8	9.6
Remainder of brain	13.3	20.3	21.6	22.4	9.1
Spinal cord	10.8	16.2	20.7	15.5	5.0
Fat	7.5	11.6	30.3	41.8	4.2
Blood	307.0	303.0	224.0	297.0	49.0

Table 3. *Distribution of triethyltin in hamster and guinea pig and the effect of perfusion*

Animals were killed 4–5 hr. after intraperitoneal injection of 10 mg. of triethyl[^{113}Sn]tin chloride/kg. body wt.

Tissue	Triethyltin ($\mu\text{moles/g. wet wt. of tissue}$)			
	Hamster		Guinea pig	
	Unperfused	Perfused	Unperfused	Perfused
Liver	161.0	124.0	342.0	275.0
Kidney	55.7	31.4	95.0	78.5
Muscle	58.0	26.0	22.7	17.8
Brain				
Cerebellum and brain stem	34.3	25.2	34.7	28.1
Remainder of brain	32.2	24.6	28.9	24.8
Spinal cord	28.9	23.1	29.3	24.4
Fat	44.5	48.7	24.0	11.2
Bile	140.0	404.0*	82.0	57.0*
Blood	7.7†		8.3†	

* Bile sampled before perfusion.

† Mean of two results.

Fig. 1. Relationship between dose and triethyltin content of rat tissues. Rats were injected intravenously, killed 2 hr. later and perfused. Each point is the mean of three results.

of triethyltin present in the blood. This is not due to the intraperitoneal route of administration, for similar results are obtained after intravenous injection (Table 4). Guinea-pig liver has two to three times more triethyltin/g. wet wt. than has rat or hamster liver, and the bile contains a high but variable content of triethyltin. Perfusion has much the same effect as it has on the distribution in rat. Larger losses were apparent in the perfused hamster, but the very high bile content (404 $\mu\text{moles/ml.}$) indicates that this particular animal may have excreted more of the triethyltin than the unperfused control (bile content 140 $\mu\text{moles/ml.}$).

Table 4. *Effect of size of dose on triethyltin distribution in guinea pig*

Animals were injected via a saphenous vein and killed after 4 hr. They were not perfused.

Dose (mg. of triethyltin chloride/kg.)	Triethyltin ($\mu\text{moles per ml. of blood or per g. wet wt. of tissue}$)			
	Blood	Liver	Kidney	Brain
1.25	0.31	70.0	7.9	1.9
	0.37	78.5	9.5	2.2
	0.41	83.0	11.5	2.5
2.5	0.58	118	15.2	3.4
5.0	1.6	199	32.8	8.03
	2.1	213	26.3	7.17

The concentration of triethyltin in guinea-pig tissues increases with the dose and no saturation levels were reached (Table 4). The liver of the guinea pig, like that of the rat, appears to have the greatest affinity for triethyltin.

Distribution of triethyltin in the blood of various species

After injection into rats, triethyltin is present in a high concentration in the blood, mainly in the erythrocytes. This is not so in the hamster or guinea pig. The distribution of triethyltin *in vitro* in the blood of other species was therefore examined. Rat erythrocytes maintain a higher cells/plasma ratio than do erythrocytes of other species studied (cf. Cremer, 1957), and when washed with saline or Krebs–Ringer phosphate (Table 5) appear unique in that virtually no triethyltin is washed out and the cells/wash fluid ratio becomes very large. All washed erythrocytes maintained a cells/wash fluid

Table 5. *Distribution of triethyltin in vitro in blood from various species and the effect of washing the erythrocytes*

To samples of fresh heparinized blood were added 0.01 vol. portions of triethyl[^{113}Sn]tin chloride. After 10 min. the blood was centrifuged and the plasma removed. Erythrocytes were washed by suspending to the volume of the original blood sample with 0.9% NaCl or Krebs-Ringer phosphate. Values are expressed as μmoles of triethyltin/ml. of packed erythrocytes, plasma or wash fluid.

	Triethyltin ($\mu\text{moles/ml.}$)		Cells/plasma ratio	Triethyltin ($\mu\text{moles/ml.}$)		Cells/wash 1 ratio	Triethyltin ($\mu\text{moles/ml.}$)		Cells/wash 2 ratio
	Cells	Plasma		Cells	Wash 1		Cells	Wash 2	
Rat	890	46	19.3	825	10.0	82.5	816	0.4	2004
Mouse	860	178	4.8	780	21.1	37.0	—	—	—
Guinea pig	670	254	2.6	645	23.5	22.6	—	—	—
Chicken	1160	157	7.4	1110	48.5	22.9	963	35.0	27.5
Rabbit	570	445	1.3	444	76.2	5.8	356	47.5	7.5
Hamster	630	445	1.4	—	—	—	—	—	—
Human	520	275	1.9	—	—	—	—	—	—

Table 6. *Binding of triethyltin to plasma from various species*

For conditions of equilibrium dialysis see the Materials and Methods section.

	Triethyltin ($\mu\text{moles/ml.}$)		Bound/free ratio
	Bound	Free	
Guinea pig	139	25.0	5.6
Mouse	110	11.9	9.3
Rat	33.3	1.25	26.6
Rabbit	24.1	5.0	4.8

Table 7. *Interaction of triethyltin and rat haemoglobin*

Each preparation was diluted to give approximately the same $E_{540\text{m}\mu}^{\text{cm.}}$ value. To 5 ml. of each were added 200 μmoles of triethyl[^{114}Sn]tin chloride in 0.1 ml. This mixture was dialysed to equilibrium as described in Materials and Methods section.

	Crystalline rat haemoglobin	Lysate of erythrocytes
$E_{540\text{m}\mu}^{\text{cm.}}$	0.475	0.490
Concn. of haemoglobin ($\mu\text{moles/ml.}$)	8.0	8.2
Concn. of triethyltin bound ($\mu\text{moles/ml.}$)	16.4	14.7
Triethyltin/haemoglobin ratio	2.0	1.8

ratio higher than the original cells/plasma ratio, suggesting that the plasma might also bind triethyltin. Equilibrium-dialysis experiments with plasma confirmed this view (Table 6).

Triethyltin binding in rat erythrocytes. On lysis of rat erythrocytes and separation of the stroma by centrifugation, more than 80% of the triethyltin is

found in the supernatant. This triethyltin is not freely diffusible and must therefore be bound to large-molecular-weight material.

Since rat oxyhaemoglobin is very insoluble and is precipitated from a lysate on standing, it may be readily purified by crystallization (Aldridge, 1951). The ability of this purified rat haemoglobin to bind triethyltin was then compared with that of a fresh lysate that had been quickly diluted to prevent loss of haemoglobin by precipitation. Both preparations bind triethyltin in proportion to their haemoglobin content, 2 moles of triethyltin combining with 1 mole of rat haemoglobin (Table 7).

Intracellular distribution of triethyltin in liver, kidney and brain

All fractions obtained from rat and guinea-pig liver contain triethyltin (Table 8), but the highest amount is found in the supernatant fraction. The specific concentration (μmoles of triethyltin/mg. of protein) is also higher in the supernatant than in other fractions and particularly so in that from guinea-pig liver. However, the triethyltin in the supernatant fractions is not freely diffusible; at equilibrium the inside/outside concentration ratio was 11:1.

In rat kidney a large proportion of the triethyltin was found in the nuclear and debris fraction (Table 9). This was not changed by modifying the homogenization procedure (gap between pestle and tube and speed of rotation). If this fraction is neglected, the distribution of the rest of the triethyltin in liver and kidney is very similar.

In contrast with rat liver and kidney, a higher percentage of triethyltin is recovered in the mitochondrial fraction from rat brain (Table 10).

The large amount of triethyltin found in the nuclear and debris fraction from liver, kidney and brain (Tables 8, 9 and 10) is undoubtedly partly due

Table 8. *Distribution of triethyltin in fractions from liver of rat and guinea pig*

For Expts. 1 and 2 rats were injected with 10 mg. of triethyl[^{113}Sn]tin chloride/kg. body wt. and killed 2 hr. after injection. The rat for Expt. 2 was without food for 24 hr. before the injection. The guinea pig was injected with 5 mg. of triethyl[^{113}Sn]tin chloride/kg. body wt. Fractionation procedures were as described in the Materials and Methods section. In parentheses the triethyltin recovered is expressed as percentages of the total recovered in the last five fractions. Recoveries of triethyltin in all fractions were 92–97% and of protein 91–98%.

Fraction	Triethyltin ($\mu\text{moles/fraction}$)			Triethyltin ($\mu\text{moles/mg. of protein}$)		
	Rat		Guinea pig	Rat		Guinea pig
	Expt. 1	Expt. 2		Expt. 1	Expt. 2	
Homogenate	936	692	1320	0.71	0.55	1.03
Nuclear and debris	285	230	194	0.59	0.45	0.37
Heavy mitochondrial	35 (6)	31 (7)	17 (2)	0.30	0.23	0.13
Light mitochondrial	27 (5)	18 (4)	19 (2)	0.62	0.57	0.43
Wash fluid	52 (9)	42 (10)	135 (12)	—	—	—
Microsomal	69 (12)	48 (11)	30 (3)	0.65	0.61	0.31
Supernatant	388 (68)	280 (67)	885 (81)	1.01	0.74	2.41

Table 9. *Distribution of triethyltin in fractions from rat kidney*

Rats were injected with 10 mg. of triethyl[^{113}Sn]tin chloride/kg. body wt. and killed 2 hr. later. Fractionation procedures were as described in the Materials and Methods section. In parentheses the triethyltin recovered is expressed as percentages of the total recovered in the last five fractions. Recoveries of triethyltin were 91 and 93% and of protein 90 and 96%.

Fraction	Triethyltin ($\mu\text{moles/fraction}$)		Triethyltin ($\mu\text{moles/mg. of protein}$)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Homogenate	253	346	0.44	0.49
Nuclear and debris	127	200	0.41	0.51
Heavy mitochondrial	13.5 (14)	16.2 (13)	0.31	0.35
Light mitochondrial	4.9 (4)	5.8 (5)	0.39	0.56
Wash fluid	10.6 (11)	11.4 (9)	—	—
Microsomal	12.5 (11)	11.4 (9)	0.51	0.56
Supernatant	60.0 (59)	78.5 (63)	0.43	0.52

Table 10. *Distribution of triethyltin in fractions from rat brain*

Rats were injected with 10 mg. of triethyl[^{113}Sn]tin chloride/kg. body wt. and killed 2 hr. later. Fractionation procedures were as described in the Materials and Methods section. In parentheses the triethyltin recovered is expressed as percentages of the total recovered in the last four fractions. Recoveries of triethyltin were 86 and 97% and of protein 88 and 92%. The wash fluid was not examined.

Fraction	Triethyltin ($\mu\text{moles/fraction}$)		Triethyltin ($\mu\text{moles/mg. of protein}$)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Homogenate	66.3	68.5	0.27	0.29
Nuclear and debris	19.1	16.7	0.42	0.41
Heavy mitochondrial	16.6 (37)	18.3 (43)	0.28	0.26
Light mitochondrial	4.6 (10)	4.1 (10)	0.26	0.27
Microsomal	9.7 (21)	6.1 (14)	0.28	0.27
Supernatant	14.9 (32)	14.0 (33)	0.24	0.24

to the presence of unbroken cells. However, in a single experiment a much purer preparation of rat liver nuclei was suspended in a solution containing triethyltin. The centrifuged nuclei gave a value of

210 for the ratio of μmoles of triethyltin/mg. of nuclear protein to μmoles of triethyltin/ $\mu\text{l.}$ of supernatant, and it is therefore probable that some component of liver nuclei has affinity for triethyltin.

Table 11. *Binding of triethyltin to various protein preparations*

Equilibrium dialysis was performed as described in the Materials and Methods section. The concentration of protein/ml. is that in the 5 ml. inside the sac, and the concentration of triethyltin added is that in the total volume (i.e. 10 ml.), since it is diffusible.

Protein	Approx. concn. (μ moles/ml.)	Triethyltin added (μ moles/ml.)	Triethyltin bound (moles/mole of protein)
Phosvitin	20	20	0
Clupein	200	20	0
Salmine	1.7 (mg./ml.)	20	0
Cytochrome c	35	20	0
Bovine plasma albumin	133	20	0.05
Horse myoglobin	71	20	0
Chymotrypsin	100	40	0
Bovine pancreatic ribonuclease	20	40	0
Haemoglobins			
Rat	20	30	2.0
Mouse	20	40	0.25
Guinea pig	240	20	0.07
Horse	18	40	0.02
Rabbit	> 20	40	0
Human	20	20	0

Equilibrium dialysis of calf thymus DNA (from British Drug Houses Ltd.) with triethyl[^{113}Sn]tin showed no binding at all, indicating that DNA is not responsible for the binding in liver nuclei.

Binding of triethyltin to other proteins

A variety of pure proteins and lysates of erythrocytes of mouse, rabbit, guinea pig, horse and human were tested to see whether they show affinity for triethyltin under conditions where rat haemoglobin would show considerable binding (Table 11). It seems likely that mouse haemoglobin has some affinity for triethyltin but it is much lower than that of rat haemoglobin. Other proteins show no such affinity.

DISCUSSION

In previous work on the distribution of triethyltin in rats (Cremer, 1957, 1958; Brody & Moore, 1962) a chemical method based on its reaction with diphenylthiocarbazon (Aldridge & Cremer, 1957) was used. This method distinguishes between triethyltin and diethyltin. There is general agreement between the chemical and radioisotopic determination of both the concentration of triethyltin in a tissue and the relationship between the concentrations in different tissues at various times after dosing. We therefore conclude that the radioactivity measured is in the form of the intact triethyltin moiety. This stability contrasts with that of tetraethyltin and diethyltin. Tetraethyltin is readily dealkylated to triethyltin by a microsomal enzyme system in liver and kidney (Cremer, 1958). After administration, diethyltin is excreted more

rapidly than triethyltin (95% in 4 days) and monoethyltin is found in urine and faeces (Bridges, Davies & Williams, 1966).

As has been shown by Cremer (1957) there is no correlation between the concentration of triethyltin and the production of pathological change. The only lesion demonstrable is oedema of the white matter of the central nervous system and yet the brain and spinal cord contain less triethyltin than other tissues.

Triethyltin is toxic to rats, guinea pigs and rabbits, yet in the rat 50% of the injected dose is attached to the haemoglobin. This combination cannot therefore be directly related to the biochemical mechanism of toxicity.

Respiration and the formation of creatine phosphate by cerebral-cortex slices are inhibited by $1\mu\text{M}$ -triethyltin whereas respiration by liver slices is unaffected (Cremer, 1957, 1961). The insensitivity of liver slices may be due to a protective effect arising from the binding of triethyltin in liver cell sap, less of the available triethyltin being in the mitochondria (only 5% of the triethyltin in liver was found in this fraction; Table 8). The intracellular distribution of triethyltin in rat brain differs from that in rat liver and kidney in that the mitochondrial fraction contains the highest proportion (40%) of the triethyltin (Tables 8, 9 and 10). Since respiration by brain slices is inhibited, brain mitochondria ought, on this basis, to have a higher specific concentration of triethyltin than liver mitochondria; but these concentrations are approximately the same (Tables 8 and 10). However, the brain mitochondrial fraction will be much more contaminated with non-mitochondrial protein than are liver

mitochondria (Whittaker, 1959) and the above comparison may therefore be misleading.

The aim of this study was to find macromolecules suitable for studying the reactivity of triethyltin. If the results of such studies are to be useful for the interpretation of the effects of triethyltin on oxidative phosphorylation, it is an important first step to show that triethyltin has specificity and does not bind to everything to the same degree. These studies establish this, for the distribution is non-uniform. Two tissues, rat blood and liver, were chosen for further study. Triethyltin interacts stoichiometrically with rat haemoglobin and this combination is very specific, since haemoglobins of other species under similar conditions bind much less. Liver supernatant fraction (particularly that from guinea-pig liver) contains the greater proportion of the triethyltin in that tissue. Current equilibrium-dialysis experiments indicate that the approximate association constants for the combination of triethyltin with rat haemoglobin and rat and guinea-pig liver supernatant fractions are 6×10^5 , 2×10^5 – 3×10^5 and $3 \times 10^6 \text{ M}^{-1}$ respectively (M. S. Rose, unpublished work). Further, a miscellaneous group of pure proteins bind very much less triethyltin under comparable conditions. It is concluded therefore that triethyltin does not have high affinity for all proteins and that rat haemoglobin and component(s) in liver supernatant fraction possess an affinity comparable with the affinity of triethyltin for sensitive sites in the system of enzymes carrying out oxidative phosphorylation. They are therefore suitable model macromolecules for further study.

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