2. The agreement in values for gelatin from chicken-leg tendon by both microbiological and ion-exchange methods is very good except for alanine, serine and isoleucine.

3. Chicken-leg-tendon gelatin is similar in composition to mammalian materials. The composition of toad-skin gelatin resembles certain fish gelatins more closely than mammalian materials, and the reverse is true for the two reptile gelatins.

4. The relationship between shrinkage temperature and hydroxyproline and serine plus threonine contents was studied. A very approximate relationship appears to exist between hydroxyproline content and shrinkage temperature.

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The Metabolism *in vitro* of Tissue Slices from Rats given Triethyltin Compounds

By JILL E. CREMER

Toxicology Research Unit, Medical Research Council Laboratories, Carshalton, Surrey

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A detailed account of the signs of the acute and chronic toxicity of alkyltin compounds in animals has been given by Stoner, Barnes & Duff (1955), who concluded that the main site of action of trialkyltin compounds appears to be in the central nervous system. Histological studies have shown that rats fed on a diet containing triethyltin hydroxide develop a specific lesion of the white matter of the brain and spinal cord (Magee, Stoner & Barnes, 1957). The lesion is characterized by an increase in the fluid content of the white matter without any apparent damage to the cells. A second characteristic is the reversibility of the lesion and recovery of animals from chronic poisoning when triethyltin is no longer administered.

The present work is an attempt to elucidate the reason for the selective action of triethyltin on the central nervous system *in vivo*, since previous biochemical studies showed that rat-liver mitochondrial preparations were highly sensitive to triethyltin (Aldridge & Cremer, 1955) and that the site of action was on the oxidative-phosphorylation mechanism.

This paper describes the respiratory activity of rat-tissue slices in the presence of triethyltin. Of the

tissues studied, only brain slices are sensitive to triethyltin and the altered metabolic activity is paralleled by the concentration of triethyltin in the slice whether it is added *in vitro* or administered to the rat before removal of the brain.

A preliminary account of the work has already appeared (Cremer, 1956).

MATERIALS AND METHODS

Tissues. The tissues were from adult albino rats, of 170–200 g. body wt., which had been fed on M.R.C. diet no. 41 (Bruce & Parkes, 1949) except where otherwise stated. In experiments where triethyltin was added to the diet triethyltin hydroxide in arachis oil was mixed with powdered M.R.C. diet no. 41. Food consumption was recorded and paired feeding carried out on control rats.

Slices of brain cortex, liver, kidney and heart were cut with a Stadie-Riggs type of tissue slicer (Stadie & Riggs, 1944). Brain subcortical white matter was prepared according to McIlwain (personal communication). Diaphragms and pieces of washed small intestine were cut into segments. Tissue slices of 0.5–0.7 mm. thickness were quickly prepared at room temperature and placed directly in Warburg flasks. Dry weights were determined by removing the slices from the flasks at the end of the incubation period and heating at 110° for 24 hr.

Each flask contained 3 ml. of Krebs-Ringer phosphate solution (Umbreit, Burris & Stauffer, 1951), with glucose 0.011M, tissue slice of 50–60 mg. wet wt. and 0.2 ml. of 20% (w/v) KOH in the centre well for absorption of CO_2 . The manometers were placed in a water bath at 37°, gassed for 5 min. with 100% O_2 , equilibrated for a further 10 min. and O_2 uptake was measured at intervals up to 75 min. Q_{O_2} is expressed as $\mu\text{l. of O}_2/\text{mg. dry wt. of tissue/hr.}$ Variations in this general procedure are given in the legends of the particular experiments.

Lactic acid was estimated by the technique of Barker & Summerson (1941) and pyruvic acid by the method of Friedmann & Haugen (1943) with the modification for increasing the sensitivity previously described (Aldridge & Cremer, 1955).

Glucose was estimated by the method of Nelson (1944), but the copper reagent used was that described by Somogyi (1945). The glucose uptake was calculated as the amount of glucose which disappeared from the medium during incubation.

Special chemicals. Glucose, lithium lactate, ethylenediaminetetra-acetic acid disodium salt (EDTA), dithiocarbazon (dithizone; British Drug Houses Ltd., Poole), sodium pyruvate (Roche Products Ltd., Welwyn) and iodoacetic acid (L. Light and Co., Colnbrook) were used.

Inhibitor. A stock solution of triethyltin sulphate $[(\text{Et}_3\text{Sn})_2\text{SO}_4; 10 \text{ mg./ml.}, 3.94 \times 10^{-2} \text{ M-triethyltin}]$ was prepared as previously described (Aldridge & Cremer, 1955).

Estimation of triethyltin. A sample of tissue weighing between 0.4 and 1.0 g. wet wt. was homogenized in 5 ml. of water with a Nelco homogenizer, at 4000 rev./min. Water (10 ml.) was added to the homogenate, followed by 5 ml. of 10% (w/v) tartaric acid. The contents of the tube were mixed thoroughly and allowed to stand for 5 min. Perchloric acid (30%, v/v; 5 ml.) was added, the contents were again thoroughly mixed, then centrifuged for 10 min. at 3300 g. A 20 ml. sample of the supernatant was taken, neutralized with 5N-NaOH (approx. 6 ml.) and 2 ml. of borate-EDTA buffer was added (19 g. of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$; 12 g. of boric acid; 4 g. of EDTA diluted to 1 l.; pH 8.4). The tubes were left to cool to room temperature. Chloroform (10 ml.) was added and the mixture extracted for 3 min. by plunging a mushroom-ended glass rod up and down. The aqueous layer was removed by suction and the CHCl_3 layer was cleared by centrifuging. The following procedures were carried out in a darkened room owing to the instability of the coloured complex to be formed. The CHCl_3 layer was transferred to a test tube (0.75 in. diam.), 3 ml. of borate-EDTA buffer was added followed by 1 ml. of dithizone

reagent [0.04% (w/v) dithiocarbazon in CHCl_3], and the contents were mixed for 1 min. with a mushroom-ended glass rod. The aqueous layer was removed by suction and the CHCl_3 layer containing the triethyltin-dithizone complex was read against a dithizone control in 2 cm. cells at a wavelength of 610 m μ , a Unicam SP. 600 spectrophotometer being used. Two drops of acetic acid (AnalaR) were then added to each tube (triethyltin does not form a complex with dithizone under acid conditions) and readings taken again. The difference value of the two readings, giving the amount of dithizone used, was compared with the amount used by a standard concentration of triethyltin. The presence of EDTA has previously been shown to prevent interference from Fe, Co, Mn and Cu ions (Aldridge & Cremer, 1957). Recoveries of triethyltin, added to tissue homogenates *in vitro*, from rat brain, liver, kidney and blood, are given in Table 1.

RESULTS

Effect of triethyltin added in vitro

Tissue slices from normal rats were incubated in Krebs-Ringer phosphate solution in the presence of triethyltin. Concentrations of 3×10^{-7} – 3×10^{-6} M appeared to affect only brain-cortical grey matter, subcortical white matter and spinal cord (Table 2). At higher concentrations several other tissues became sensitive to triethyltin, particularly kidney and diaphragm. At the lowest concentrations of triethyltin used there is a slight stimulation of O_2 uptake of brain slices and spinal cord. The concentration causing stimulation of brain cortex and subcortical white matter appeared to be highly critical, since in two other experiments with the same dilution of triethyltin either no effect or slight inhibition of O_2 uptake was observed.

A more constant feature of the effect of triethyltin is the lowered pyruvate and increased lactate levels, a phenomenon previously noted by Aldridge & Cremer (1955) in rat-brain brei preparations, for which no satisfactory explanation could be given. Stotz & Bessey (1942), studying the effect of thiamine deficiency in pigeons on the pyruvic and lactic acid levels of blood, showed that although the absolute values for each acid varied for the controls

Table 1. *Recovery of triethyltin added to rat tissues in vitro*

Recoveries are expressed as the mean percentage recovery with the standard error of the mean (S.E.M.) and the number of determinations (in parentheses).

	Range of total triethyltin added ($\mu\text{g.}$)					
	3–5		6–10		16	
	Average recovery (%)	S.E.M.	Average recovery (%)	S.E.M.	Average recovery (%)	S.E.M.
Brain	77	± 1.54 (5)	75	± 2.83 (8)	78	± 3.97 (6)
Liver	76.6	± 4.9 (4)	73.7	± 2.74 (8)	79.1	± 5.46 (6)
Kidney	82.9	± 6.27 (5)	77	± 1.93 (8)	79.8	± 2.95 (6)
Blood	—	—	85	± 2.88 (6)	88	± 4.8 (4)

Table 2. *Effect of triethyltin added to rat-tissue slices in vitro*

For brain-cortex, liver and kidney slices the details were as described in Methods. Triethyltin, where present, was added before the addition of the slices. After incubation for 75 min., slices were removed for dry wt. determination, and 3 ml. of 18% (w/v) trichloroacetic acid (TCA) was added to the remaining flask contents. After centrifuging, lactic and pyruvic acids were determined in samples of the supernatant. For brain white matter, spinal cord, heart, small intestine and diaphragm flasks of 7 ml. total capacity were used. Flasks were prepared in pairs, each containing 1 ml. of Krebs-Ringer phosphate with glucose 0.011 M, triethyltin where indicated, and tissue samples of approx. 25 mg. wet wt. After incubation for 75 min. the tissue samples were removed and 1 ml. of 18% (w/v) TCA was added to each flask. The contents of the paired flasks were pooled for pyruvic and lactic acid determinations. Q_{O_2} is expressed as μ l. of O_2 /mg. dry wt./hr., lactic and pyruvic acids as μ g. found in the total fluid volume after TCA addition and pooling of samples where indicated. Temp., 37°.

Tissue	Concn. of triethyltin (M)	Q_{O_2}	Lactic acid (μ g.)	Pyruvic acid (μ g.)	Lactic acid/pyruvic acid
Brain cortex	Nil	11.5	236	12.5	18.9
	2.8×10^{-6}	4.3	531	3.5	151
	9.3×10^{-7}	9.0	405	3.8	106
	3.1×10^{-7}	13.5	301	6.6	45.5
Brain white matter	Nil	5.9	296	12.6	23.5
	1.3×10^{-6}	3.9	376	4.9	77
Spinal cord	Nil	4.05	90	5.3	17
	2.6×10^{-6}	5.06	152	3.4	45
	9.8×10^{-7}	4.8	113	3.1	36
Liver	Nil	6.2	58	8.3	7.0
	1.5×10^{-6}	6.4	80	6.4	12.5
	4.9×10^{-6}	7.1	79.5	9.8	8.1
Kidney	Nil	14.1	59.5	6.7	8.9
	2.0×10^{-6}	11.2	145	3.9	36.5
	6.6×10^{-6}	11.8	55.4	4.0	14
Heart	Nil	7.6	125	11	11.4
	4.9×10^{-6}	8.3	160	13	12.3
Diaphragm	Nil	6.9	43	4	10.8
	1.3×10^{-6}	6.5	111	2	55
	4.4×10^{-6}	6.3	50	2.5	20
Small intestine	Nil	5.2	272	4	68
	4.9×10^{-6}	4.7	302	5.6	54

Table 3. *Effect of triethyltin and potassium cyanide on aerobic and anaerobic glycolysis of brain and liver slices*

Each flask contained 3 ml. of Krebs-Ringer phosphate with 0.011 M-glucose and triethyltin or KCN as indicated. Average wet wt. of tissue slice was 56 mg. When KCN was added the 0.2 ml. of 20% (w/v) KOH normally used in the centre well was replaced by 0.2 ml. of a mixture of equal parts of N-KCN and N-KOH (Krebs, 1935). For aerobic experiments the gas phase was O_2 and for anaerobic experiments N_2 was used. After incubation for 75 min. at 37° slices were removed and 3 ml. of 18% (w/v) TCA was added. Samples of the flask contents were taken for lactic and pyruvic acid determinations. The values are expressed as total μ g. of each acid/flask.

Inhibitor concn. (M)	Aerobic			Anaerobic	
	Q_{O_2}	Lactic acid (μ g.)	Pyruvic acid (μ g.)	Lactic acid (μ g.)	Pyruvic acid (μ g.)
Brain-cortex slices					
Nil	12.4	186	11.4	253	1.5
Triethyltin (1.3×10^{-6})	7.4	440	5.5	126	<1
KCN (1.1×10^{-6})	3.7	575	1.9	305	<1
Liver slices					
Nil	6.2	58	8.3	167	<1
Triethyltin (1.3×10^{-6})	6.4	80	6.4	167	<1
KCN (3.3×10^{-6})	3.9	196	2.4	266	1.3

from experiment to experiment, the ratio of lactic to pyruvic acid was constant, but in thiamine-deficient animals there was an abnormal ratio. A similar way of presenting the relationship of lactic to pyruvic acid in normal and triethyltin-treated slices has been adopted here, and for brain and spinal-cord slices this ratio is increased in the presence of triethyltin (Table 2).

The anaerobic glycolysis of normal brain slices was studied (Table 3). The levels of lactic acid were low compared with values obtained by McIlwain (1956) and earlier workers, but Beloff-Chain, Catanzaro, Chain, Masi & Pocchiari (1955), using [^{14}C]glucose, found that anaerobic glycolysis in rat-brain slices was low, only 7% of the added glucose being utilized compared with 50% under aerobic conditions.

The present results show that the ratio of lactic acid to pyruvic acid under anaerobic conditions was appreciably higher than in an aerobic system owing mainly to the low pyruvate level. The effect of triethyltin on brain slices respiring under aerobic conditions could be explained by this compound causing the system to become anaerobic. The effect of triethyltin on the activity of brain and liver slices under anaerobic conditions was examined (Table 3) and lactic acid production by brain slices only was found to be inhibited. Cyanide, which resembles triethyltin in increasing the lactic acid:pyruvic acid ratio under aerobic conditions, was without effect on anaerobic glycolysis. It has previously been shown that rat-brain lactic dehydrogenase is not inhibited by triethyltin (Aldridge & Cremer, 1955). The effect of iodoacetic acid on brain slices described by Heald (1953) was confirmed and the lowering of

both pyruvate and lactate levels under aerobic conditions by this compound indicated that triethyltin does not act by inhibiting a specific enzyme of the glycolytic chain such as triose phosphate dehydrogenase. To determine the effect of triethyltin on the uptake of glucose from the medium by brain-cortex slices, the amount of tissue added to each flask was doubled. Results of typical experiments under aerobic and anaerobic conditions are given in Table 4. They show that in the aerobic system the uptake of glucose measured for the control could be accounted for as O_2 consumed and lactic acid found in the medium. In the presence of triethyltin the O_2 uptake was lowered but the glucose uptake was increased, and this increase could be accounted for as lactic acid. Under anaerobic conditions both the glucose uptake and the lactate level were lowered by triethyltin. Since triethyltin has been shown to be a potent inhibitor of oxidative phosphorylation by brain mitochondria (Aldridge, unpublished work), the altered metabolism of brain slices caused by this compound appears to be more likely due to alterations in their levels of adenosine phosphates and pyridine nucleotides than to direct inhibition of known enzymes.

Effect of transferring brain slices from a medium containing triethyltin to fresh medium. To determine whether the effect of triethyltin added to brain slices *in vitro* was reversible, slices were incubated in a medium containing triethyltin for 45 min., then transferred to fresh medium and incubated for a further 45 min., the O_2 uptake, pyruvate and lactate levels being determined for each period. Results from a typical experiment are given in Table 5,

Table 4. *Effect of triethyltin on glucose uptake by brain-cortex slices*

Each flask contained 3 ml. of Krebs-Ringer phosphate with 0.011 M-glucose and 2.2×10^{-6} M-triethyltin where indicated. Average wet wt. of tissue slices was 137 mg. Values are expressed as $\mu\text{moles/flask/75 min.}$

	Aerobic			Anaerobic	
	O_2 uptake (μmoles)	Lactic acid formed (μmoles)	Glucose utilized (μmoles)	Lactic acid formed (μmoles)	Glucose utilized (μmoles)
Control	14.9	5.0	5.0	7.2	3.5
Triethyltin	6.47	12.4	6.85	4.52	2.23

Table 5. *Effect of transferring brain-cortex slices from Ringer medium containing triethyltin to fresh Ringer medium*

Components of the flasks were as given in Methods with 6.9×10^{-7} M-triethyltin where indicated. After incubation for 45 min. one slice from each pair was transferred to a flask containing 3 ml. of Krebs-Ringer phosphate with 0.011 M-glucose. After gassing for 4 min. with O_2 , readings of O_2 uptake were taken up to 105 min. Slices were removed for dry wt. determination and 3 ml. of 18% (w/v) TCA was added to each flask. Oxygen uptake is expressed as $\mu\text{l. of O}_2/\text{mg. dry wt.}$

System	O_2 uptake (25–45 min.)	Lactic acid/ pyruvic acid	O_2 uptake (85–105 min.)	Lactic acid/ pyruvic acid
Control (not transferred)	4.0	—	4.0	14.4
Triethyltin (not transferred)	3.3	—	1.5	94.0
Control (transferred)	4.7	23	3.3	23.0
Triethyltin (transferred)	3.7	68	1.5	57.0

which shows that the inhibition of O_2 consumption is not reversed by transferring the slices. Although the lactic acid:pyruvic acid ratio is less than in slices remaining in a medium containing triethyltin it is still higher than the control.

Distribution of triethyltin in rat tissues

The method for estimating triethyltin in biological material, described under Methods, appeared sufficiently reliable for it to be used in the present study to determine the pattern of distribution and approximate concentrations of triethyltin in the tissues and organs of rats given triethyltin, in an attempt to correlate the biologically effective concentrations *in vivo* and *in vitro*.

Rats placed on a diet containing 20 p.p.m. of triethyltin hydroxide for 5 days, followed by 10 p.p.m. for 12 weeks, were killed; various organs were removed and analysed individually for their triethyltin content. Results given in Table 6 show that although triethyltin could be detected in all tissues examined it was not evenly distributed, the highest concentrations being found in blood and liver. The concentrations of triethyltin found in spleen, kidney, heart and brain were lower. The

compound does not accumulate in the central nervous system more than in other tissues.

Results given in Table 6 are mean values from three determinations expressed as $\mu\text{g.}$ of triethyltin/g. wet wt. of tissue, correction being made for the percentage recoveries given in Table 1. The tissues were washed thoroughly before analysis and no allowance has been made for the blood content. From a sample of rat blood to which 26 $\mu\text{g.}$ of triethyltin had been added, 23 $\mu\text{g.}$ was recovered from the erythrocytes plus stroma and none was detected in the plasma. This pattern of distribution is different for rabbit blood where, in a similar experiment, 9 $\mu\text{g.}$ was recovered from the erythrocyte fraction and 17 $\mu\text{g.}$ from the plasma.

Activity of tissue slices from rats given triethyltin

Rats given a diet containing triethyltin do not gain in weight, owing primarily to a low intake of food (Stoner *et al.* 1955). In the present study, pair-fed controls have been used. Rats fed with triethyltin which were used for the experiments described in Table 7 were from the same group of rats used for the distribution data given in Table 6. For comparative purposes the activities of slices prepared from normal and pair-fed controls are also given (Table 7). Histological examination of the brain and spinal cord of the rats on a diet containing triethyltin showed that the lesion of the white matter previously described by Magee *et al.* (1957) was well established. Brain-cortex slices from rats fed with triethyltin showed an altered metabolism *in vitro*, whereas liver and kidney slices had an activity similar to those prepared from pair-fed control animals. Magee *et al.* (1957) showed that the lesion of the white matter had disappeared in rats which had been placed again on a normal diet for 4 months. The activity of brain-cortex slices prepared from two rats again put on a triethyltin-free diet for 12 days was normal (Table 7), and no triethyltin could be detected in the various organs analysed.

Activity of tissue slices from rats given a single dose of triethyltin. Since the brain-cortex slices with an altered metabolism *in vitro*, recorded in Table 7, were prepared from rats in which a marked lesion of

Table 6. *Concentration of triethyltin in tissues of three rats given a diet containing the compound*

Diet contained 20 p.p.m. of triethyltin hydroxide for 5 days followed by 10 p.p.m. for 12 weeks. Average total consumption was 11 mg. of triethyltin hydroxide/rat. For analytical procedure see text and Methods. Total blood vol. has been taken as 7% and skeletal muscle as 50% of body wt. Average wt. of rats when killed was 230 g.

Tissue	Mean total wet wt. (g.)	Triethyltin	
		Concn. ($\mu\text{g./g.}$ wet wt., corr.)	Total ($\mu\text{g./tissue}$)
Blood	—	17.6	283.0
Brain	1.78	2.9	5.2
Heart	0.82	5.2	4.3
Spleen	0.68	5.5	3.8
Liver	9.90	19.8	196.0
Kidney	1.77	6.4	11.3
Skeletal muscle	—	1.46	206.0
		Total	709.6

Table 7. *Activity of tissue slices from rats given a diet containing triethyltin*

For details see text and Table 2. Values are averages for three rats in each group.

Type of rat	Brain cortex		Liver		Kidney	
	Q_{O_2}	Lactic acid/pyruvic acid	Q_{O_2}	Lactic acid/pyruvic acid	Q_{O_2}	Lactic acid/pyruvic acid
Normal	10.2	27	6.0	7.2	16	7.1
Pair-fed control	10.5	24	6.5	16.2	14.8	9.9
Fed with triethyltin	6.6	82	6.0	11.2	14.4	6.9
Placed back on normal diet for 12 days	12.3	19	7.4	4.0	17.8	4.7

the white matter had developed, the activity of slices from rats given single doses of triethyltin was examined. Rats given an intraperitoneal injection of triethyltin sulphate (10 mg./kg.) were killed after 2 hr. and slices from various tissues were prepared. Their activities are given in Table 8. The dose used was lethal; rats not killed at 2 hr. died within 2-3 days. Again only nervous tissue was affected.

Uptake of triethyltin by brain and liver slices

Table 9 shows the amount of triethyltin found in brain-cortex and liver slices after they had been incubated in the Krebs-Ringer phosphate medium containing triethyltin. Under the conditions of the experiments the ratio of the volume of the medium to the wet weight of tissue was approximately 50. With both brain and liver slices, when triethyltin was added at two different concentrations in the medium the percentage taken up by the slices was the same. Similar results were obtained for brain white matter and kidney slices. If the triethyltin found in the slice after incubation was calculated as the molar concentration in the tissue fluid of the slice (taking the tissue fluid as 80% of the wet

weight and making no allowance for any swelling of the slice in the medium) there was a concentration of triethyltin approximately 17 times greater in the slice than in the medium added at the beginning of the experiment. This factor has been used to calculate the amount present in slices from a known concentration added in the medium where the concentrations of triethyltin were too low to come within the sensitivity of the analytical method. The effect of several known metabolic inhibitors and lowered temperature were examined (Table 9) in an attempt to determine whether the uptake of triethyltin by tissue slices required energy or whether it was due to physical-adsorption processes. Only a lowered temperature caused a reduction in the amount of triethyltin found in the slice.

Comparison between dose, symptoms and activity of brain slices

Rats were given intraperitoneal injections of triethyltin sulphate in the doses stated in Table 10, and killed 2 hr. later. The brains were rapidly removed, the activity of slices prepared from one half was determined and triethyltin analysis carried out on the other half. Triethyltin was also

Table 8. *Activity of tissue slices from rats given a single dose of triethyltin*

For details see text and Table 2. Values are averages for two rats in each group.

Tissue	Normal		Triethyltin injected intraperitoneally	
	Q_{O_2}	Lactic acid/ pyruvic acid	Q_{O_2}	Lactic acid/ pyruvic acid
Brain cortex	11.5	19.0	5.2	108.0
Brain white matter	5.9	23.5	3.6	97.0
Liver	6.2	7.0	6.2	6.6
Kidney	14.1	8.9	15.7	9.8

Table 9. *Distribution of triethyltin between Krebs-Ringer phosphate and tissue slices and the effect of metabolic inhibitors*

Flasks were prepared in duplicate; each contained 3 ml. of Krebs-Ringer phosphate with 0.011 M-glucose. Triethyltin, and metabolic inhibitors where indicated, were added together before the addition of the tissue slices. The centre well contained 0.2 ml. of 20% KOH, except where KCN was used, when it contained 0.2 ml. of a mixture of 10 ml. of N-KCN and 1 ml. of N-KOH (Krebs, 1935). After incubating for 60 min. at 37° (except where otherwise stated) the slices were removed, rinsed quickly with water, blotted, homogenized in pairs and analysed for triethyltin. Total triethyltin added was determined for each experiment. *R* is the quotient: molar concn. of triethyltin in the tissue fluid (80% of tissue wet wt.) of the slices/molar concn. added in the medium.

Inhibitor concn. (M)	Concn. of triethyltin added (10^{-5} M)	Concn. of triethyltin found in slices (10^{-4} M)			
		Brain	<i>R</i>	Liver	<i>R</i>
—	5.3	10.4	20	8.8	16.5
—	1.77	2.7	15	2.5	14
Iodoacetic acid (10^{-2})	7.25	12.5	17	14.5	20
Malonate (10^{-2})	6.2	11.7	19	14.7	24
KCN (10^{-3})	6.95	11.8	17	12.2	17.5
Temp. lowered to 12°	7.75	6.7	8.7	—	—
Temp. lowered to 12°	8.46	—	—	7.25	8.6

determined in blood, liver and kidney samples. The results given in Table 10 show there is an agreement between the dose given, the intensity of the signs of poisoning, the amount of triethyltin present in the tissues and the lowered metabolic activity of brain-cortex slices.

Comparison of the activity of tissue slices with triethyltin added in vitro and in vivo. The values given in Table 11 were obtained in an attempt to correlate the activity of tissue slices prepared from rats given triethyltin with normal slices to which triethyltin had been added. The concentrations of triethyltin added to tissue slices *in vitro* have been calculated as described above (Table 9). The concentrations *in vivo* after intraperitoneal injection or feeding have been calculated as the molar concentration of triethyltin, assuming all the triethyltin found in the brain on analysis to be in the tissue fluid. Results of the activity of brain-cortex slices show that the effect of triethyltin on O_2 uptake and the lactic acid:pyruvic acid ratio was similar whether the triethyltin was added *in vitro* or given to the rat *in vivo*. Triethyltin had no effect on liver slices. The

data were obtained from rats given intraperitoneal injections of triethyltin sulphate as described in Tables 8 and 10, except where otherwise stated.

Effect of 2:4-dinitrophenol on brain slices in the presence of triethyltin

Many workers have stimulated brain-cortex slices with 2:4-dinitrophenol (DNP), and Ghosh & Quastel (1954) in particular have shown that the stimulated portion of the O_2 consumption is more sensitive to certain narcotics than is the unstimulated portion. The effect of triethyltin on DNP stimulation of brain slices has been determined; the results are given in Table 12. In the experiments recorded, DNP was added to brain slices after they had been respiring in the presence of triethyltin so that a comparison could be made with slices prepared from rats injected with triethyltin.

Experiments in which slices were first stimulated with 2.7×10^{-5} M-DNP for 30 min. and then had 8×10^{-7} M-triethyltin added, showed that the stimulation of O_2 uptake was abolished by triethyltin. Since brain slices in the presence of

Table 10. *Comparison of dose, symptoms and activity of brain slices*

For details see text and Table 2. Values are averages for two rats in each group. Symbols are used to indicate the presence and intensity of the signs of poisoning in the rats at the time of killing: —, rats appeared to behave no differently from the controls; +, rats showed minimum movement and ruffled hair; ++, rats showed also signs of muscular weakness, particularly of the hind limbs; + + +, rats were prostrate.

Dose of triethyltin (mg./kg.)	Symptoms	Triethyltin (μ g./g. or ml.)				Brain cortex			
		Brain	Liver	Kidney	Blood	Q_{O_2}	Lactic acid	Pyruvic acid	Lactic acid/pyruvic acid
Nil	—	0	0	0	0	12.6	186	11.4	16
2	—	2	12.2	3.8	10.8	12.9	206	8.9	23
4	+	1.6	10.6	4.5	14.6	14.7	223	8.3	27
8	++	3.3	17.6	6.3	48	10.3	389	5.4	72
12	+++	4.2	25	9.0	95	5.9	368	4.0	92

Table 11. *Comparison of the activity of slices with triethyltin added in vitro and in vivo*

For details of triethyltin concentrations see text.

Tissue	Concn. of triethyltin in tissue fluid (M)		Q _{O₂}	Lactic acid/ pyruvic acid
	Added <i>in vitro</i>	Intraperitoneal injection <i>in vivo</i>		
Brain cortex	—	—	12.6	16
	4.8 × 10 ⁻⁵	—	4.3	151
	5.3 × 10 ⁻⁶	—	13.5	45
	—	2.5 × 10 ⁻⁵	5.9	92
	—	1.7 × 10 ^{-5*}	6.6	82
	—	9.7 × 10 ⁻⁶	14.7	27
Liver	—	—	6.2	7.0
	2.5 × 10 ⁻⁴	—	6.4	12.5
	8.3 × 10 ⁻⁵	—	7.1	8.1
	—	1.5 × 10 ⁻⁴	6.2	6.6
	—	1.2 × 10 ^{-4*}	6.0	11.2
	—	—	—	—

* Rats were fed with triethyltin; see Tables 6 and 7.

Table 12. *Effect of 2:4-dinitrophenol on rat-brain-cortex slices in the presence of triethyltin*

Flask contents were as described in Table 2. A volume (0.1 ml.) of 2:4-dinitrophenol ($8.35 \times 10^{-5}M$) was added from the side arm after incubation for 45 min. and readings of O_2 uptake were continued until 85 min.

Concn. of triethyltin in tissue fluid ($10^{-5}M$)		DNP added	O_2 uptake ($\mu l./mg.$ dry wt.)		Change in O_2 uptake (%)
Added <i>in vitro</i>	Intraperitoneal injection <i>in vivo</i>		15-45 min.	55-85 min.	
—	—	—	6.2	6.4	+3
—	—	+	4.5	7.2	+60
1:1	—	—	5.6	3.0	-40
1:1	—	+	4.3	2.3	-47
—	2.5	—	3.2	2.8	-12
—	2.5	+	3.6	2.7	-25

triethyltin could not be stimulated by DNP, and since in fact the inhibition of O_2 uptake increased with time to the same extent as with triethyltin alone, it seemed of interest to see whether brain slices prepared from rats injected with triethyltin could be stimulated by DNP. Rats were killed 2 hr. after an intraperitoneal injection of triethyltin (12 mg./kg.), and brain slices were prepared in the usual way and treated as described in Table 12. The molar concentration of triethyltin in the brain-tissue fluid of the injected rats was calculated as described for Table 11 by using the value of triethyltin found in the brain by analysis given in Table 10. Similar results to those given in Table 12 were obtained with slices of brain white matter; again neither brain slices with triethyltin added *in vitro* nor slices prepared from rats injected with triethyltin could be stimulated by DNP. All attempts to stimulate rat-liver slices with DNP failed. Results of other workers have shown small and variable stimulation (McCord, 1934; Muntwyler, 1935; Thomson, 1950), and Niemeyer & Figueroa (1955) have demonstrated that the initial glycogen content of liver slices influences their response to DNP.

DISCUSSION

The finding from experiments in which triethyltin was added to rat-tissue slices *in vitro* that only brain slices were sensitive to this compound was of particular interest, since previous work with rat-liver mitochondrial preparations had shown that triethyltin was a potent inhibitor of oxidative phosphorylation (Aldridge & Cremer, 1955). Aldridge (unpublished work) has since shown that triethyltin at low concentrations inhibits the oxidative phosphorylation of rat-brain mitochondria. A difference in sensitivity to certain drugs between whole-cell and fractionated-cell preparations is well known. Nevertheless, a difference of over 20-fold in the sensitivity to triethyltin between rat-brain and -liver slices remains striking, since both tissues

are inhibited by similar concentrations when mitochondrial preparations are used.

Wenner & Weinhouse (1956) have confirmed that liver slices utilize only a small percentage of added glucose compared with non-hepatic tissues, and Gey (1956) has recently shown that rat liver contains ten times as much glucose as brain per g. of tissue. Brain tissue is known to be highly dependent on glucose so that a possible explanation of the sensitivity of this tissue to triethyltin could be that this compound in some way prevents glucose, added externally to brain slices, from reaching metabolic sites within the slice. However, this seems unlikely from the results given in Table 4 where, under aerobic conditions, triethyltin in fact increased the glucose uptake of brain-cortex slices. The simultaneous increase in lactic acid indicates that triethyltin interferes with the final oxidative pathway rather than the initial phosphorylation of glucose.

Since tissue slices other than brain were unaffected by triethyltin at low concentrations it seemed possible that under the experimental conditions the actions of drugs on certain tissues were not readily detectable. However, $5 \times 10^{-5}M$ -KCN (Table 3) altered the metabolic activity of both brain and liver slices in a similar manner, and recently Coke & Mija (1956) have shown that rat-liver and -jejunum slices are markedly inhibited by certain antispasmodic drugs in conditions identical with those used in the present work. Webb & Elliott (1951), also using similar experimental conditions, found that pentobarbital inhibited the oxygen uptake and acid production of both brain and kidney slices.

Since measurements of the distribution of triethyltin between medium and tissue slices showed that similar amounts were taken up by brain, liver and kidney slices, there appeared to be an equal chance of inhibiting each type of tissue. Results from a single experiment, in which triethyltin was determined in nuclear, mitochondrial and micro-

somal plus supernatant fractions prepared by differential centrifuging techniques (Aldridge, 1957) from the liver of a rat injected with triethyltin, showed that triethyltin was present in each fraction. However, it is not possible to assess how far redistribution of triethyltin occurs during the preparation, and therefore the distribution pattern of triethyltin within different tissues *in vivo* remains obscure.

The mechanism whereby triethyltin exerts its effect on brain slices is still far from clear. The present results appear only to confirm the conclusions reached in previous work *in vitro* with rat-liver mitochondria and brain brei preparations (Aldridge & Cremer, 1955). Although at low concentrations triethyltin caused a slight increase in oxygen uptake of brain slices the results were variable and a more common feature was inhibition. It seems unlikely therefore that triethyltin acts like DNP, particularly since DNP added to brain slices in the presence of triethyltin did not show an increased oxygen uptake. Furthermore, V. H. Parker (unpublished work) has found that triethyltin prevents the stimulation of adenosine triphosphatase activity by DNP.

Triethyltin appears to be a stable substance in animal tissues when compared with many other drugs, thus rendering it a convenient compound with which to relate action to concentration. Distribution data given in Table 6 show that the selective action of triethyltin on the central nervous system cannot readily be explained by the compound becoming concentrated in this tissue, although from the chemical properties of triethyltin one might expect it to be preferentially distributed in lipid material, in which the central nervous system is particularly rich. However, within 1–2 hr. after an intraperitoneal injection triethyltin can be detected in the brain in sufficient amounts to account for its effect on metabolic activities. Triethyltin is concentrated by brain slices when this substance is added to the medium, and, as shown in Table 11, it has been possible to remove brains from rats obviously ill from triethyltin poisoning to determine the concentration of triethyltin in the whole brain, and to demonstrate an altered metabolic activity *in vitro* almost identical with that of normal slices to which triethyltin has been added to give a final concentration in the slice similar to that in the brains of the injected rats. Although the correlation between activity and concentrations *in vitro* and *in vivo* appears to be a direct one, it has not been ascertained where the triethyltin is located or how much is active, and calculations have only been made assuming it to be entirely in the total tissue fluid. Nevertheless, such a close agreement between the active concentrations is of interest, since similar comparisons with other drugs

have sometimes shown wide discrepancies (Buchel & McIlwain, 1950; Butler, 1950; Sutherland, Hine & Burbridge, 1956). With these other drugs, however, closer agreement between the active concentrations has been obtained when the concentration *in vitro* has been taken as that which inhibited the portion of brain-slice oxygen consumption stimulated electrically or by K^+ ions (Ghosh & Quastel, 1954; McIlwain, 1953), whereas triethyltin, at low concentrations, inhibits the unstimulated portion. The altered activity of brain slices from rats suffering from triethyltin poisoning was similar whether the rats had been maintained on a diet containing triethyltin and showed a marked oedema of the white matter, or whether they were killed 2 hr. after an intraperitoneal injection of triethyltin. Since slices of both grey and white matter were affected, although only white matter shows pathological damage, it is not clear whether the biochemical lesion observed is related to the pathological one, and work on high-energy phosphate levels and turnover rates is being done in these laboratories to help to elucidate the relationship, particularly since no cellular damage has been detected histologically. The present results appear to indicate that the altered activity of brain slices from triethyltin-treated rats is due directly to the presence and concentration of triethyltin in the tissue, thus causing a similar behaviour to that of normal slices with triethyltin added *in vitro*. The fact that DNP did not stimulate brain slices from triethyltin-injected animals would seem to support this idea.

The lowered oxygen consumption of brain slices prepared from rats given triethyltin is very striking, in some instances being as low as 50 % of the normal rate (Table 8). It is possible that brain metabolism *in vivo* is not lowered to this extent and that this low activity of brain slices is in some way accentuated by the technique used. Some support to this idea is given by a few (unpublished) results for brain slices, obtained with a different technique, devised by Rodnight & McIlwain (1954) and Huston & Martin (1955). Slices were suspended in a moist atmosphere on silver-gauze mats and oxygen consumption was recorded by the conventional Warburg method. Brain slices, examined in this way, prepared from triethyltin-injected rats, had rates of oxygen consumption similar to those of control slices, although when duplicate slices were placed in Krebs–Ringer phosphate solution they showed a marked inhibition of oxygen uptake as described above. The outstanding difference between the two results indicates the difficulty of comparing data from tissue slices *in vitro* with possible changes *in vivo*. Although both techniques have obvious limitations it still seems pertinent that, with the procedure used throughout the present study, brain slices removed

from rats given triethyltin show a lowered metabolism *in vitro*, whereas slices prepared from liver and kidney, which contain relatively high concentrations of triethyltin, do not differ from the controls. Many earlier attempts (summarized by Quastel, 1955) to prepare brain slices from animals treated with drugs, particularly narcotics, and to demonstrate an altered metabolism *in vitro* have failed. It therefore seems of special interest that a substance such as triethyltin can produce so striking an effect.

The following comparisons between the clinical symptoms and tissue-slice activity of triethyltin-poisoned rats can be made. In both instances triethyltin appears to act selectively on the central nervous system. When rats which have been fed on a diet containing triethyltin are again given a normal diet, the oedema of the white matter disappears and the activity of brain slices returns to a more normal level. Peruzzo & Forni (1953) have made similar comparisons with chlorpromazine. They also demonstrated that the oxygen consumption of brain slices only was inhibited, both when chlorpromazine was added *in vitro* or when slices were prepared from injected rats. A further similarity between triethyltin and chlorpromazine is that both inhibit oxidative phosphorylation of rat-liver mitochondria (Andrejew & Rosenberg, 1956; Berger, Strecker & Waelsh, 1956), whereas liver slices are unaffected. Both compounds *in vivo* have a tranquilizing effect on rats and bring about a marked lowering of body temperature (Stoner *et al.* 1955; Peruzzo & Forni, 1953). There is nothing known about the effect of repeated doses of chlorpromazine on brain structure.

SUMMARY

1. The effect of triethyltin on the respiratory activity of rat-tissue slices has been examined. Brain slices are the most strongly inhibited.

2. Brain slices prepared from rats given triethyltin show a lowered metabolism *in vitro*. Liver and kidney slices do not differ from controls.

3. Data on triethyltin distribution in the rats show that the highest concentrations are in erythrocytes and liver.

4. *In vitro*, brain, liver and kidney slices take up triethyltin from the medium to produce a concentration greater in the tissues than in the medium.

5. When the amount of triethyltin found in whole brain *in vivo* and in slices *in vitro* is expressed as the concentration in the tissue fluid, there is a close correlation between the concentrations which are effective *in vivo* and *in vitro*.

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