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TRIALKYLLEADS AND OXIDATIVE PHOSPHORYLATION: A STUDY OF THE ACTION OF TRIALKYLLEADS UPON RAT LIVER MITOCHONDRIA AND RAT BRAIN CORTEX SLICES

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Abstract—A study has been made of the actions of trialkylleads upon processes associated with oxidative phosphorylation in rat liver mitochondria and rat brain slices. Trialkyltins and trialkylleads have similar actions upon these processes. This similar biochemical behaviour is consistent with similar chemical properties. However, their toxic effects in animals differ and these cannot be explained.

PREVIOUS studies have shown that trialkyltin compounds act both *in vivo* and *in vitro* as the intact organotin molecule.[1, 2] A homologous series of trialkyltins from methyl to hexyl inhibit oxidative phosphorylation somewhere in the energy transferring chain between electron transport and the formation of ATP.[1, 3–5] In addition, they stimulate adenosinetriphosphatase to varying degrees, but the degree of stimulation never approaches that produced by 2:4-dinitrophenol.[3] The extent to which they stimulate adenosinetriphosphatase does not run parallel to their inhibition of the various reactions associated with oxidative phosphorylation. It is, therefore, not certain that the stimulation of adenosinetriphosphatase is related to their inhibitory power against oxidative phosphorylation.[3]

The respiration of slices of cerebral cortex is inhibited by triethyltin at concentrations similar to those which are effective against liver mitochondria.[6] It was shown that the most sensitive index of altered metabolism was the ratio of the lactate and pyruvate concentrations present at the end of these experiments. The synthesis of creatine phosphate is also prevented by triethyltin and this occurs with lower concentrations than are required to inhibit respiration.[7]

It has been shown that triethyllead inhibits the respiration of brain slices and increases the lactate–pyruvate ratio in a similar way to triethyltin.[8] In the present work this initial observation has been extended by a study of the action of a homologous series of trialkylleads upon both brain slices and liver mitochondria.

METHODS

Rat liver mitochondria

The mitochondria were isolated with a Potter–Elvehjem-type homogenizer, with a smooth glass tube and Perspex pestle with a total clearance of 0·02 in.[9, 3] The construction of the homogenizer has been described.[10] Respiration of the mitochondria

was measured in the presence and absence of apyrase.[11] The substrate used was pyruvate (0·01 M) with fumarate (1·0 mM) and sufficient apyrase was added to hydrolyse 800 μg atom P/hr. Oxidative phosphorylation was measured according to Aldridge,[3] and adenosinetriphosphatase as described by Aldridge and Stoner.[11] The ^{32}P-ATP exchange was determined in a medium (3 ml) containing ATP (3 mM), KCl (0·1 M), $MgCl_2$ (14 mM), EDTA (1·0 mM), glycylglycine (16·7 mM) inorganic phosphate (3·3 mM) 1 μc of ^{32}P (equivalent to 200,000 recorded counts/min in the counting system used) and sucrose (30 mM). The pH was adjusted to 6·8 with KOH. The procedure for incubation and determination of the radioactivity in the separated adenine nucleotides is given in detail by Aldridge and Threlfall.[4] In all of these experiments the specific radioactivity of the exchangeable β- and γ-phosphorus atoms of ATP was approximately 40 per cent of the equilibrium value. The specific activity of the β-phosphorus atom of ADP was 80–100 per cent that of the β- and γ-phosphorus atoms of ATP.

Brain slices

Rat brain cortex slices were prepared and used as described by Cremer,[6] and creatine phosphate synthesis measured according to Cremer.[7]

Analytical methods

The following methods were used: phosphate, Fiske and Subbarow[12]; protein by the biuret method of Robinson and Hogden[13] as modified by Aldridge[9]; lactic acid, Barker and Summerson[14]; pyruvic acid, Friedemann and Haugen[15, 1]; free and total creatine, Eggleton, Elsden and Gough[16], as modified by Ennor and Rosenberg[17]; trialkyltins and trialkylleads, Aldridge and Cremer[18].

Inhibitors

Trimethyllead and triethyllead chlorides were supplied by Associated Ethyl Co. Ltd. and tri-*n*-propyllead and tri-*n*-butyllead chlorides by Ethyl Corporation; New York. These compounds are unstable to light and were stored cold and in the dark. Fresh solutions were prepared in dimethylformamide for each experiment, and 0·03 ml of these solutions was added to each flask. In most experiments a concentration of 1% dimethylformamide in the incubation mixtures was never exceeded. Because dimethylformamide interfered in the colorimetric method for creatine, a final concentration of 0·03% dimethylformamide was not exceeded in some experiments.

2:4-Dinitrophenol was used as a stock solution neutralized with KOH to approximately pH 7.

Special chemicals

The following chemicals were obtained from the sources indicated: sodium salt of AMP, disodium salt of ATP (Sigma Chemical Co., St. Louis, Mo., U.S.A.), sodium pyruvate, glycylglycine (Roche Products Ltd.).

RESULTS

Liver Mitochondria

Respiration

Using pyruvate as substrate the respiration of rat liver mitochondria is increased three- to four-fold by the addition of potato apyrase.[9] The additional oxygen

uptake must therefore be associated with the phosphorylation of adenine nucleotide. Like trialkyltins, the trialkylleads inhibited the oxygen uptake induced by apyrase. The effect of a range of concentrations of trimethyllead is shown in Fig. 1. Trimethyllead (6 μM) inhibited by 50 per cent the oxygen uptake induced by apyrase. In the absence of apyrase the oxygen uptake at first increased and was then inhibited as the

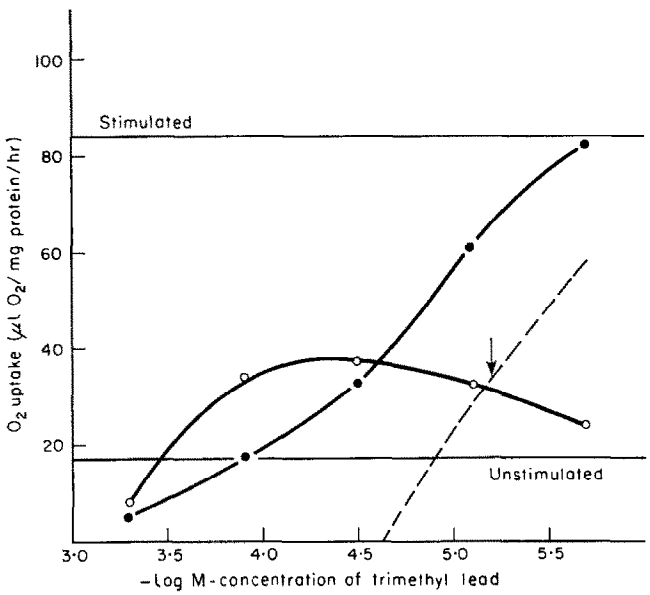

FIG. 1. The effect of trimethyllead on the stimulated and unstimulated oxidation of pyruvate by mitochondria. ○ Unstimulated oxidation: ● Oxidation in the presence of apyrase.[3] Mitochondria were added to the flasks containing the medium and trimethyllead; readings were taken from 10–30 min after placing the flasks in the bath at 37 °C. The broken line is the difference between the two curves. The pI_{50} (5·15) for the inhibition of the stimulation of oxygen uptake by apyrase has been derived from this line and indicated by the arrow.

concentration of trimethyllead increased. All the trialkylleads studied behaved in a qualitatively similar way. There are, however, quantitative differences between the various trialkylleads and these are given in Tables 1 and 2. All the trialkylleads at the lower concentrations used stimulated oxygen uptake in the absence of apyrase (Table 1). They formed a continuous series in activity, the concentrations which produced maximal stimulation decreasing from trimethyl to tri-*n*-butyllead. The degree of stimulation was the same for trimethyl, triethyl and tri-*n*-propyllead but was lower for tri-*n*-butyllead. At higher concentrations, all the trialkylleads inhibited respiration. The concentrations which inhibited the stimulating effect of apyrase on oxygen uptake are given in Table 2: trimethyllead was the least active, the other three trialkylleads were equally active.

Oxidative phosphorylation

When oxidative phosphorylation was measured using hexokinase and glucose to trap ATP formed, the trialkyltins were shown to be potent inhibitors of oxidation and phosphorylation.[3] Trialkylleads also inhibited oxidative phosphorylation and the

concentrations necessary to cause inhibition were very similar to those required to inhibit oxygen uptake induced by apyrase (Table 2). The degree of inhibition of oxygen and phosphate uptakes was very close and over the range 0–50 per cent inhibition, oxygen uptake was only 10 per cent less inhibited than phosphate uptake. Any lowering of the P/O ratio is therefore small.

TABLE 1. THE ACTION OF TRIALKYLLEADS UPON THE UNSTIMULATED RESPIRATION AND ADENOSINETRIPHOSPHATASE OF MITOCHONDRIA

Concentration of trialkyllead (μM)	O_2 uptake degree of inhibition or stimulation	Adenosinetriphosphatase degree of stimulation
Concentrations producing maximum stimulation of respiration		
Methyl, 30	2·1 (4·8)	7·6 (16·2)
Ethyl, 4·0	2·0 (3·7)	9·0 (26·5)
n-Propyl, 1·0	2·1 (4·8)	6·3 (16·8)
n-Butyl, 0·8	1·4 (4·5)	7·4 (16·8)
Concentrations producing inhibition of respiration		
Methyl, 500	0·43 (4·8)	7·6 (16·2)
Ethyl, 100	0·41 (3·7)	10·5 (26·5)
n-Propyl, 20	0·26 (4·8)	6·5 (16·8)
n-Butyl, 25	0·11 (4·5)	7·0 (16·8)

The degree of activation or inhibition is the activity in the presence of trialkyllead divided by the control activity. The figures in brackets indicate the degree of stimulation obtained for maximal O_2 uptake in the presence of apyrase or for maximal adenosinetriphosphatase in the presence of 2:4-dinitrophenol (30 μM).

TABLE 2. THE CONCENTRATIONS OF TRIALKYLLEADS PRODUCING 50 PER CENT INHIBITION OF SOME PROCESSES ASSOCIATED WITH OXIDATIVE PHOSPHORYLATION

Concentrations producing 50 per cent inhibition (μM).

Trialkyllead	Oxidation induced by apyrase	P uptake	ATPase in presence of 2:4-dinitrophenol	^{32}P-ATP exchange
Methyl	5·6	7·0	3·0	16
Ethyl	0·35	0·50	0·9	1·4
n-Propyl	0·45	0·40	0·25	0·45
n-Butyl	0·35	0·45	0·25	0·40

Mitochondrial adenosinetriphosphatase

The adenosinetriphosphatase of the mitochondrial preparation used in this work was low (approx. 0·5 μg atom P/mg protein/hr at 37 °C). This was stimulated by 2:4-dinitrophenol (30 μM) to approx. 10 μg atom P/mg protein/hr. Since, qualitatively, the series of trialkylleads were similar in their action, results from a typical experiment with trimethyllead are shown in Fig. 2. Adenosinetriphosphatase activity was increased, though never to the extent produced by 2:4-dinitrophenol. The stimulation of adenosinetriphosphatase by 2:4-dinitrophenol was inhibited by trimethyllead.

The concentrations of the trialkylleads necessary to inhibit the stimulation of adenosinetriphosphatase by 2:4-dinitrophenol are similar to those required to inhibit respiration and phosphorylation (Table 2). When the respiration of unstimulated mitochondria is increased by trialkylleads, the adenosinetriphosphatase is also increased (Figs. 1 and 2 and Table 1). However, a further increase in the concentrations

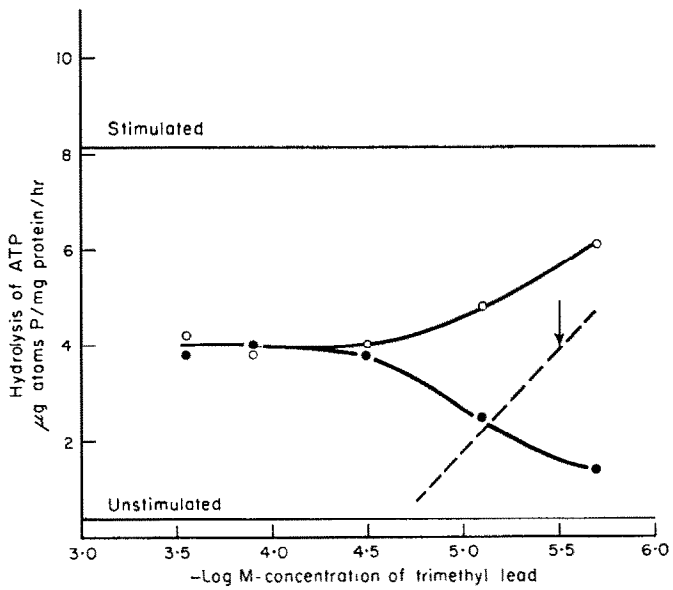

FIG. 2. The influence of trimethyllead on latent and 2:4-dinitrophenol-stimulated adenosinetriphosphatase. Mitochondria were added to the vessels containing the medium and the trimethyllead. ● Without 2:4-dinitrophenol: ○ in the presence of 33 μM 2:4-dinitrophenol. Inorganic phosphate was determined at 0 °C and 10 min after the addition of the mitochondria. The broken line is the difference between the two curves. The concentration for the inhibition of the stimulation of adenosinetriphosphatase by 2:4-dinitrophenol has been derived from this line (pI_{50} = 5·50; I_{50} = 3·0 μM) and indicated by the arrow.

of the trialkylleads leads to an inhibition of oxygen uptake whereas the adenosinetriphosphatase is undiminished and sometimes increased. At this stage, however, the suspensions of mitochondria have become much more transparent and it is obvious that gross swelling has occurred. Under these circumstances, it is unlikely that oxygen uptake with pyruvate as a substrate would occur whereas adenosinetriphosphatase activity would certainly be present.

^{32}P-*ATP exchange*. Trialkyltins inhibit the ^{32}P-ATP exchange reaction of liver mitochondria.[4] The trialkylleads also inhibit this reaction, the effective concentrations being similar to those inhibiting other reactions associated with oxidative phosphorylation (Table 2).

Succinic oxidase. Under conditions when 1 μg atom of oxygen is taken up for every μmole of succinate oxidized, triethyltin does not produce any inhibition.[1] However,

when succinate oxidation is coupled to phosphorylation, respiration is inhibited by triethyl and tri-*n*-butyltin.[3, 19] In Table 3 are shown the results of some experiments where the oxidation of succinate was carried out in a hypotonic medium containing cytochrome-*c*, but in the absence of nucleotide. Under these conditions, 1 μg atom of oxygen was taken up for the oxidation of 1 μmole of succinate. Very high concentrations of trialkylleads were required to produce appreciable inhibition of this system

TABLE 3. THE EFFECT OF TRIALKYLLEAD COMPOUNDS UPON THE OXIDATION OF SUCCINATE

Concentration of trialkyllead (μM)		Inhibition of succinate oxidation (%)	Concentration of trialkyllead giving the same inhibition of pyruvate oxidation (μM)
Methyl,	1000	29	3·1
	100	0	—
Ethyl,	1000	39	0·35
	100	25	0·2
n-Propyl,	1000	89	4·5
	100	22	0·11
	10	11	0·056
n-Butyl,	1000	89	5·0
	100	18	0·09
	10	17	0·08

The medium contained $MgCl_2$ (14 mM), K salt of EDTA (1 mM), potassium phosphate (15 mM), cytochrome-*c* (500 μg), succinate (30 mM) and the pH was adjusted to 6·75 with KOH.

(Table 3). The concentration of each trialkyllead which produced the same amount of inhibition of oxygen uptake during measurements of oxidative phosphorylation with pyruvate as a substrate are included in Table 3: these are 100–1000 times smaller. This means in most cases, that during the oxidation of pyruvate, even if all the trialkyllead added were present within the mitochondria, the concentrations would not be high enough to produce appreciable inhibition of succinic oxidase in the intact mitochondria (the calculations of these concentrations within the mitochondria have been based on a ratio of mitochondrial wet wt to volume of medium of 1:160. The mitochondrial wet wt was calculated from mitochondrial protein using the results of Werkheiser and Bartley[20]).

Slices of Brain Cortex

Glucose metabolism

The effect of the series of trialkyllead compounds on the metabolism of glucose by brain cortex slices is given in Table 4 and in Fig. 3. The changes brought about by all four compounds were of a similar pattern. At the higher range of concentrations oxygen uptake was inhibited, lactic acid increased and pyruvic acid decreased. At

lower concentrations there was stimulation of oxygen consumption. However, within this overall pattern there were differences specific to each compound. For instance, although trimethyllead was the least active it caused the greatest stimulation of oxygen uptake: also tributyllead lowered the level of pyruvate more than the other three compounds.

It may be seen from the curve given in Fig. 3 for the action of triethyllead on oxygen consumption that there was an abrupt change from inhibition to stimulation over a small change in concentration from pC 6·0 to 6·3. The points given for this portion of the curve were from separate experiments so that a slight experimental error in attaining the exact final concentration of the lead compound could account for the different response given by brain slices at a theoretical concentration of pC 6·2. Similar curves were obtained for the other three compounds although the inhibition of oxygen uptake by tributyllead was a more gradual process.

Creatine phosphate synthesis

It is well known that during the preparation of brain slices creatine-phosphate falls to zero level but during incubation in a suitable medium containing glucose or

TABLE 4. THE EFFECT OF TRIALKYLLEAD COMPOUNDS ON OXYGEN UPTAKE AND CHANGES IN LACTATE/PYRUVATE RATIO OF RAT BRAIN CORTEX SLICES

Trialkyllead	Concentration (μM)	QO_2	Lactic acid (μg/flask)	Pyruvic acid (μg/flask)	Ratio: *Lactate* pyruvate
—	—	12·8	178	10·6	17
Methyl	26·3	7·6	540	6·8	80
	8·8	18·8	240	13·1	18
	1·5	14·6	262	12·1	22
Ethyl	6·3	4·1	578	3·9	148
	0·7	12·2	618	7·4	84
	0·11	15·5	185	12·6	15
n-Propyl	5·7	4·8	500	2·6	192
	0·96	8·9	582	3·9	149
	0·24	15·5	225	7·0	32
n-Butyl	5·3	6·8	347	1·6	217
	3·3	10·1	307	3·9	79
	1·2	15·5	369	5·8	64
	0·61	12·7	239	8·4	28

Each flask contained 3 ml of Krebs–Ringer phosphate solution,[26] with 0·011 M glucose, trialkyllead compound as indicated and tissue slice (average wet wt. 56 mg). The centre-well contained 0·2 ml of 20% (w/v) KOH and the gase phase was O_2. After incubation for 75 min at 37 °C 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. QO_2 is expressed as μl of O_2/mg dry wt./hr, lactic and pyruvic acids as μg found in the total fluid volume after TCA addition and removal of the tissue slice.

pyruvate as substrate it is re-synthesized to levels approaching those found in intact brain. The effect of the trialkyllead compounds on the synthesis of creatine phosphate by brain slices *in vitro* is given in Table 5. The values given are for the first or outside slice cut from each cerebral hemisphere.

A second, or inside, slice was also used in each experiment but although the oxygen consumption was the same for both slices the amount of creatine-phosphate synthesized in the inside slice was consistently lower (1·38 μmoles/g) than in the outside slice. This difference is probably related less to the anatomical structure of the brain than to the amount of damage done to the cells during the slicing procedure.

Two concentrations of each trialkyllead compound were selected; one which inhibited oxygen consumption approximately 50 per cent and the other which stimulated oxygen consumption or, in the case of tributyllead which had no effect. In the presence of the higher concentration of each trialkyllead compound creatine-phosphate synthesis was completely inhibited. At the lower concentrations creatine-phosphate

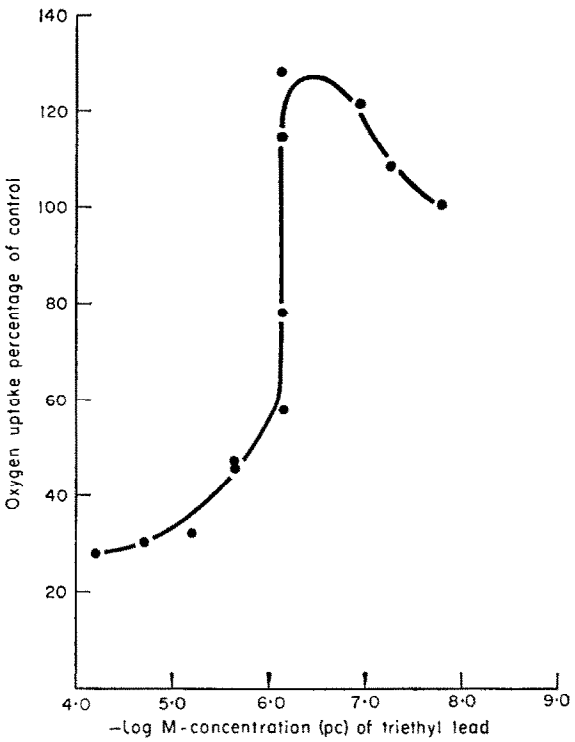

FIG. 3. The effect of triethyllead on oxygen uptake of brain slices. Conditions were as described in Table 4.

synthesis was still inhibited. Triethyllead was less active in this respect than the other three compounds. Values have also been given in Table 5 of the amount of creatine found in the medium at the end of 75 min incubation. When oxygen uptake was inhibited by the trialkyllead compounds there was a leakage of creatine from brain slices into the medium. There was no leakage when oxygen uptake was stimulated.

TABLE 5. THE EFFECT OF TRIALKYLLEAD COMPOUNDS ON CREATINE AND CREATINE PHOSPHATE LEVELS IN BRAIN SLICES

Trialkyllead compound	Concn. (μM)	O_2 uptake		Creatine phosphate		Creatine (μmoles/g)		
		μl/mg wet wt/hr	% of control	μmoles/g	% of controls	Slice	Medium	Total
—	—	2·27	100	2·13	100	6·1	4·6	10·7
Methyl	23·2	1·29	57	0·107	5	4·35	6·15	10·5
	7·7	2·73	120	0·374	18	5·57	5·15	10·72
Ethyl	1·26	1·12	48	0	0	3·43	7·03	10·46
	0·42	2·76	122	1·66	78	6·12	4·12	10·24
Propyl	1·5	1·15	51	0	0	4·22	5·43	9·65
	0·66	2·58	114	0·328	15	5·1	4·9	10·0
Butyl	5·0	1·64	72	0·381	18	3·6	7·9	11·5
	2·0	2·2	97	0·855	40	4·84	4·52	9·36

Conditions were identical to those described in Table 4 except that at the end of the 75 min incubation period the tissue slice was removed for analysis of free and total creatine and 1·0 ml of 30% (w/v) TCA was added to the medium followed by analysis for total creatine.

The results obtained for the percentage inhibition of creatine phosphate synthesis by rat brain cortex slices have been compared with the percentage inhibition of phosphate uptake during oxidative phosphorylation by rat liver mitochondria. They agree well and in no case does the difference in the percentage inhibition of the two systems by a given concentration of trialkyllead differ by more than 20 per cent.

DISCUSSION

The purpose of this work was to discover if there were differences in the action of the trialkyltins and trialkylleads on a variety of biological systems. The conclusion of this work is that the similarity between the two groups of compounds is very great, though there are one or two quantitative rather than qualitative differences.

The following chemical properties of both trialkyltins and trialkylleads are similar:

(1) Ionize to give univalent ions.

(2) Lack of affinity for SH groups.[1, 8]

(3) Lack of affinity for EDTA.[18, 8]

(4) Produce yellow complexes with dithizone with identical absorption complexes.[18]

As shown in Table 6, the differences are minor quantitative ones. Trialkyllead compounds distribute more in favour of water when shaken with chloroform than the corresponding tin compounds and the trialkyllead compounds seem to complex more readily with dithizone.

TABLE 6. DISTRIBUTION OF VARIOUS TRIALKYLTIN AND LEAD COMPOUNDS BETWEEN CHLOROFORM AND PHOSPHATE BUFFER

	Distribution coefficient ($CHCl_3$: buffer)	
	Tin compound	Lead compound
Trimethyl	0·020	0·0024
Triethyl	3·17	0·215
Tri-*n*-propyl	>20	10
Tri-*n*-butyl	>20	>20
	Distribution coefficient ($CHCl_3$ + dithizone: borate buffer + EDTA)	
Trimethyl	0·6	>40

Distribution coefficients ($CHCl_3$: phosphate buffer) were determined by mixing 5 ml $CHCl_3$ containing trialkyltin or trialkyllead with 5 ml phosphate buffer (0·067 M, pH 7·4). The compound remaining in the $CHCl_3$ layer was then determined.[18] The distribution coefficient ($CHCl_3$ + dithizone: borate buffer pH 8·4 + EDTA) was calculated from the difference between the calibration curves obtained using various amounts of aqueous phase. The dithizone concentration and the composition of the borate buffer were as previously described.[18]

The action of trialkyltins and trialkylleads against various biochemical systems are similar in the following ways:

(1) Increase of respiration of rat liver mitchondria.

(2) Inhibition of the stimulation of respiration by apyrase or hexokinase and glucose.

(3) Activation of some adenosinetriphosphatase of liver mitochondria. The extent of the activation was always less than half that obtained by 2:4-dinitrophenol.

(4) Inhibition of the stimulation of mitochondrial adenosinetriphosphatase by 2:4-dinitrophenol.

(5) Inhibition of the ^{32}P-ATP exchange.

(6) Inhibition of oxidative phosphorylation with only small changes in P/O ratio.

(7) Inhibition of respiration of slices of rat brain cortex.

(8) Inhibition of creatine phosphate formation by rat brain cortex slices.

As with the trialkyltins, with trialkylleads the concentrations that caused inhibition were of the same order for the various reactions of liver mitochondria. The agreement is not so close as for the trialkyltins and no certain reason may be given for this. It may, however, be associated with problems of penetration into the mitochondria; the trialkyltins distribute less in favour of water than their corresponding trialkyllead compounds. One difference is that, for some trialkyltins, there is a close agreement between the stimulation of adenosinetriphosphatase and the stimulation of respiration. As the concentration of trialkyltin increases, so adenosinetriphosphatase and respiration increase. Further increase in concentration leads to a decrease in both activities.[3] As shown in Figs. 1 and 2, this does not occur with the trialkylleads. As the concentration of trimethyllead increases, adenosinetriphosphatase and respiration increases. A further increase in concentration leads to a decrease in respiration but often, in contrast, an increase in adenosinetriphosphatase. Preliminary experiments have indicated that this adenosinetriphosphatase is probably associated with a profound swelling of the mitochondria.[21] With the series of trialkyltins, such a swelling and increase in adenosinetriphosphatase have been demonstrated with tri-*n*-butyltin but only at concentrations higher than those required to inhibit oxidative phosphorylation.[3]

Although there is a close similarity between the series of trialkyltin and trialkyllead compounds in regard to their action on certain biochemical processes studied *in vitro*, they do not bring about the same signs of poisoning in animals. There is some similarity in their effect on the behaviour of rats, between trimethyltin and triethyllead and also between animals given triethyltin and tri-*n*-propyllead.[2, 22] These similarities may be connected with similar physical properties (cf. Table 6). However, the outstanding feature of an oedematous swelling of the white matter of the brain and spinal cord obtained in rats given triethyltin and tri-*n*-propyltin,[23, 24, 2] has never been found in rats poisoned with triethyllead[8] or tri-*n*-propyllead.[25] The fact that trimethyltin does not produce oedema of the white matter[2] suggested that the distribution between lipoidal material and aqueous phase might be an important factor (cf. Table 6 for distribution coefficients between chloroform and water). However, since the distribution coefficient of tri-*n*-propyllead is intermediate between that of triethyltin and tri-*n*-propyltin, this property alone cannot be the deciding factor for producing oedema. Therefore, there does seem to be a qualtitative difference in the biological properties between trialkyltin and trialkyllead compounds. We have no explanation for this.

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