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The Decrease of Mitochondrial Substrate Uptake Caused by Trialkyltin and Trialkyl-lead Compounds in Chloride Media and its Relevance to Inhibition of Oxidative Phosphorylation

By DAVID N. SKILLETER
Biochemical Mechanisms Section, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, U.K.

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1. In a 100mM-KCl medium (pH 6.8) containing ATP, triethyltin (1 μM) causes a decrease in the uptake of pyruvate, malate, citrate or β-hydroxybutyrate by rat liver mitochondria, but no decrease is observed in a 100mM-KNO_3 medium. This response is not modified by the presence of rotenone in the incubation medium. 2. In the KCl medium at least 1 μM-triethyltin is required to cause maximum inhibition of pyruvate uptake. 3. Trimethyltin, tributyltin and the trialkyl-lead analogues at 1 μM, to varying degrees, also cause a decrease in pyruvate uptake by mitochondria only in the KCl medium. 4. Triethyltin stimulates resting respiration of mitochondria with all the substrates tested in the KCl medium but not in the KNO_3 medium, yet this stimulation of O_2 uptake occurs under conditions when substrate uptake is decreased. 5. In contrast, both O_2 uptake during state 3 respiration and ATP synthesis when linked to the oxidation of pyruvate, malate or citrate are strongly inhibited by 1 μM-triethyltin in a KCl medium, but O_2 uptake and ATP synthesis during the oxidation of β-hydroxybutyrate are only slightly affected. In a KNO_3 medium O_2 uptake and ATP synthesis linked to the oxidation of all substrates are only slightly affected. 6. The relevance of the decrease in substrate uptake by mitochondria caused by triethyltin in a KCl medium to the greater sensitivity of various mitochondrial functions observed *in vitro* is discussed. It is concluded that decrease of matrix substrate content is probably not the major cause of the greater sensitivity of oxidative phosphorylation to triethyltin in a KCl medium observed previously.

The inhibition of oxidative phosphorylation by triethyltin *in vitro* has been shown to be due to at least two actions. One action, independent of the nature of the incubation medium, is the binding of triethyltin to an inhibitory site in the mitochondrial membrane (Aldridge & Street, 1970, 1971). However, when mitochondria are suspended in a predominantly halide medium a much greater sensitivity to triethyltin is exhibited compared with that observed in non-halide media such as nitrate (Rose & Aldridge, 1972). Under these conditions up to 80% inhibition of ATP synthesis can be obtained with 1 μM-triethyltin (Aldridge & Street, 1964), a concentration which would only cause about 10% of the binding sites to be saturated (Aldridge & Street, 1971).

Mitochondria have been shown to normally be impermeable to Cl^- and NO_3^- ions (Chappell & Robinson, 1968). The studies of Selwyn *et al.* (1970) and Stockdale *et al.* (1970) showed that in a KCl medium triethyltin facilitates Cl^- entry into the mitochondrial matrix by mediating a Cl^-/OH^- exchange. Harris *et al.* (1973) confirmed the postulate that, for pyruvate at least, the Cl^- ion was able to become a competitive anion thus displacing substrate taken up into the matrix. Triethyltin does not appear to mediate a NO_3^-/OH^- exchange in a KNO_3 medium.

By using the conditions employed by Aldridge and co-workers (Aldridge & Street, 1970, 1971; Rose & Aldridge, 1972), the present work examines the effect of triethyltin and several other trialkyltin and trialkyl-lead compounds on the uptake of either pyruvate, malate, citrate or β-hydroxybutyrate by rat liver mitochondria in Cl^- and NO_3^- media in an attempt to determine if substrate depletion might explain the greater sensitivity of oxidative phosphorylation to triethyltin in Cl^- media observed by Rose & Aldridge (1972). It provides evidence that, whereas to various extents the different trialkyltin and trialkyl-lead compounds decrease substrate uptake by mitochondria only in the Cl^- medium, the decrease in substrate content is probably not sufficient to be the major explanation for the characteristics observed in halide media. Further, in contrast with the Cl^--dependent triethyltin inhibition of oxidative phosphorylation linked to the oxidation of pyruvate, malate and citrate when β-hydroxybutyrate is the oxidizable substrate, relatively little Cl^- sensitivity is

observed. These observations are discussed with reference to the actions of triethyltin on different mitochondrial functions.

Materials and Methods

Materials

Triethyltin sulphate was prepared from triethyltin hydroxide supplied by the Tin Research Institute, Greenford, Middx., U.K. Trimethyltin acetate was purchased from BDH Chemicals Ltd., Poole, Dorset, U.K., and tributyltin acetate, trimethyl-lead acetate, triethyl-lead acetate, tributyl-lead acetate and triethylgermanium acetate were obtained from Institute for Organic Chemistry, Utrecht, Holland. Trimethyltin acetate and triethyltin sulphate were dissolved in water and the remaining trialkyl compounds in dimethylformamide. The following were purchased: ATP, ADP, lactate dehydrogenase, malate dehydrogenase, β-hydroxybutyrate dehydrogenase and citrate lyase from Boehringer (London) Corp., London W5 2TZ, U.K.; rotenone (recrystallized from ethanol by addition of water), glycylglycine, citric acid and $HClO_4$ (60%) from BDH Chemicals Ltd.; sodium pyruvate, L-malic acid and DL-β-hydroxybutyrate from Sigma Chemical Co., St. Louis, Mo., U.S.A.; Versiluble F50 silicone oil from Jacobson Van Den Berg and Co., London W3 7RN, U.K.; radiochemicals from The Radiochemical Centre, Amersham, Bucks., U.K. All other reagents were of A.R. grade.

Methods

Preparation of mitochondria. Rat liver mitochondria were prepared as described by Aldridge & Street (1971), and suspended in 0.3M-sucrose at a protein concentration of 20–25mg/ml.

Incubations. Mitochondria (0.15ml) were added to an incubation medium (2.85ml) containing substrate (5mM), KCl (100mM) or KNO_3 (100mM), $Mg(NO_3)_2$ (14mM), EDTA (1mM), ATP (3mM) and glycylglycine (16.7mM) at pH6.8. Where indicated, P_i (6.7mM), rotenone (1.1μM) and triethyltin (1μM) were also present before the addition of mitochondria. The incubation was carried out at 37°C with shaking in open 25ml beakers. After 5min, samples were withdrawn for determination of mitochondrial substrate content.

Determination of mitochondrial substrate content. Samples (5×0.2ml) of each incubation mixture were centrifuged through silicone oil into $HClO_4$ (1.5M) as described by Harris & Van Dam (1968), in Eppendorf Centrifuges model 3200 and 0.4ml capacity polypropylene centrifuge tubes. Five portions (20μl) of the $HClO_4$ extracts of incubation mixtures were combined and transferred directly to cuvettes for determination of substrate concentration (see below). Measurement of substrate content from incubations over the range 4–10min did not vary significantly, indicating that steady-state concentrations had been reached.

The true substrate uptake by the mitochondrial matrix was obtained by correcting the measured value for the substrate present in the intermembrane space by using the volume of the sucrose-accessible space as measured by Harris & Van Dam (1968). The sucrose-inaccessible space (matrix volume) was also measured by the same technique. Table 1 gives the values used in the subsequent calculations, and indicates that in both Cl^- and NO_3^- media the sucrose-accessible spaces appeared to decrease and the sucrose-inaccessible spaces increase in the presence of rotenone (1.1μM). Values for substrate uptake are quoted in nmol/mg of mitochondrial protein, which, in agreement with Harris *et al.* (1973), is a more consistent way of expressing the results than in concentration terms.

Measurement of substrates. Pyruvate, L-malate, citrate and DL-β-hydroxybutyrate were measured enzymically with lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (EC 1.1.1.37) coupled with glutamate–oxaloacetate transaminase (EC 2.6.1.1), citrate lyase (EC 4.1.3.6) coupled with malate dehydrogenase, and β-hydroxybutyrate dehydrogenase respectively, essentially as described by

Table 1. *Values of sucrose-accessible and sucrose-inaccessible spaces of mitochondria in different media*

The media used were those described in the Materials and Methods section. Values are expressed as means ±S.E.M. from six determinations. Spaces were obtained from the addition of [U-^{14}C]sucrose (30μl, 25μCi/ml) and ^3H_2O (15μl, 0.5mCi/ml) to the incubation medium, followed by analysis as described by Harris & Van Dam (1968).

Medium	Sucrose-accessible space (μl/mg of mitochondrial protein)		Sucrose-inaccessible space (μl/mg of mitochondrial protein)	
	Rotenone absent	Rotenone present	Rotenone absent	Rotenone present
KCl	3.70±0.06	3.38±0.03	0.29±0.17	0.57±0.27
KCl+triethyltin (1μM)	3.84±0.33	3.66±0.19	0.33±0.10	0.53±0.18
KNO_3	3.33±0.14	3.29±0.18	0.38±0.06	0.89±0.21
KNO_3+triethyltin (1μM)	3.61±0.10	3.23±0.09	0.54±0.26	0.67±0.21

Bergmeyer (1963). For β-hydroxybutyrate only the D-β-hydroxybutyrate isomer can be measured enzymically, but both isomers are taken up by the mitochondria (Harris & Manger, 1969); thus the measured amount was doubled to obtain a value for the uptake of both isomers. All measurements were made on a Cary 14 spectrophotometer at 25°C and the amount of substrate present was calculated by reference to the absorbance changes observed for known substrate standards.

Measurement of ATP synthesis and O_2 uptake. ATP synthesis over 2min at 37°C was determined by measuring P_i uptake under conditions of state 3 respiration (Chance & Williams, 1956). The media used were the same as those described under 'Incubations' except that 3mM-ADP was used instead of ATP and 1.33mM-P_i was also present. The reaction was stopped by addition of 7ml of ice-cold $HClO_4$ (0.725M) and the P_i uptake determined by the method of Fiske & Subbarow (1925) as described by Aldridge & Street (1971).

O_2 uptake was measured polarographically at 37°C in media described under 'Incubations' containing either ATP (3mM) or ADP (3mM) and P_i (1.33mM).

Mitochondrial protein was determined by the method of Robinson & Hogden (1940) as modified by Aldridge (1962).

Measurement of radioactivity. When determining the volumes of the mitochondrial spaces and also the uptake of [1-^{14}C]pyruvate by mitochondria the radioactivity in the $HClO_4$ extracts was measured in 15ml of scintillator comprising 0.6% 2,5-diphenyloxazole and 12.2% 2-ethoxyethanol in toluene. Counting efficiency was determined by means of addition of internal standards to the samples.

Results

Effect of medium anions

The effect of triethyltin on the uptake of various NAD-linked substrates by mitochondria in the presence of ATP was examined at pH6.8 in media in which the bulk constituent was either KCl or KNO_3. The effect was also investigated when rotenone was present in the incubation medium. The substrates examined were pyruvate, malate and citrate as examples of a monocarboxylic, dicarboxylic and tricarboxylic acid respectively and for which passage into the mitochondrial matrix is obligatory for their oxidation via the tricarboxylic acid cycle. In addition, β-hydroxybutyrate was studied, since this substrate is metabolically distinct from the other substrates tested in that the dehydrogenase is associated with the inner membrane of mitochondria (Werner & Neupert, 1972). The implication, therefore, is that transport of this substrate into the matrix may not be

Table 2. *Effect of anions in the medium on substrate uptake by mitochondria*

The media used were those described in the Materials and Methods section in which the bulk constituent was either KCl or KNO_3 (100mM). All substrates were present at a concentration of 5mM, and where indicated P_i (6.7mM), rotenone (1.1 μM) and triethyltin (1 μM) were included in the medium. Values for substrate uptake are corrected for substrate present in the intermembrane space (see under 'Methods') and expressed as means±S.E.M. from four determinations.

Medium	Substrate uptake (nmol/mg of mitochondrial protein)							
	Pyruvate*		L-Malate		Citrate		DL-β-Hydroxybutyrate	
	Control	+Triethyltin	Control	+Triethyltin	Control	+Triethyltin	Control	+Triethyltin
KCl	12.0±0.72	3.3±0.14	24.9±1.86	8.0±0.12	34.0±5.4	15.1±2.7	32.2±0.91	12.0±0.57
KCl+rotenone	21.6±0.66	10.9±1.34	30.2±1.41	12.4±0.66	35.5±4.2	16.3±3.1	27.7±0.98	10.4±0.82
KNO_3	10.1±0.82	11.2±0.64	28.8±1.70	27.5±1.53	33.2±2.3	34.4±2.9	33.0±0.66	32.1±1.00
KNO_3+rotenone	16.0±0.51	16.3±0.92	32.1±1.33	30.1±1.81	33.5±2.5	32.3±2.6	31.6±0.59	31.3±0.81
KCl+P_i	4.5±1.37	1.1±0.49	16.4±1.10	6.3±0.76	14.1±2.1	11.2±1.8	12.2±0.62	6.4±0.48
KCl+P_i+rotenone	11.4±0.56	5.0±0.81	12.1±0.98	2.6±0.43	16.6±1.9	10.2±1.9	9.7±0.58	4.3±0.51

* The medium also contained 1mM-fumarate (Aldridge & Street, 1971).

necessary for its oxidation, although recent studies indicate that β-hydroxybutyrate does have to enter the matrix to be oxidized (Land & Clark, 1974).

The results in Table 2 show that in the Cl^- medium 1 μM-triethyltin caused a marked fall in the uptake of all the substrates tested. Triethyltin had no effect in the NO_3^- medium. Further, the fall in the mitochondrial matrix content of any individual substrate caused by triethyltin was virtually the same in both the presence and the absence of rotenone, indicating that the inhibition of the electron-transfer chain at the site of the NADH dehydrogenase could not modify this action of triethyltin. For pyruvate, and to a lesser extent malate, however, the actual uptake in the presence of rotenone was greater. No satisfactory explanation for this can be given, except that it may be related to the increased matrix volume measured in the presence of rotenone.

If P_i was added to the Cl^- medium at a concentration similar to that of the substrates present, the matrix substrate content measured was greatly decreased in both the presence and the absence of rotenone and triethyltin. This is consistent with P_i acting as a competitive anion with the substrate for the matrix pool, as observed by Papa *et al.* (1971).

In a KCl medium the lowest substrate content of the matrix was obtained with pyruvate in the presence of triethyltin (Table 2). If the relevant values quoted in Table 2 are converted into matrix concentrations by using the values in Table 1, concentrations of 10 and 3 mM are obtained in the absence and the presence of 6.7 mM-P_i respectively. The relatively large variation in the estimation of matrix volumes experienced in these studies does mean that the calculated concentrations could be in error by about 50%. However, even taking this into account the present data do suggest that the matrix concentration of all the substrates tested would be adequate to facilitate maximum oxidation rates.

Effect of triethyltin concentration on pyruvate uptake

Fig. 1 indicates that in a KCl but not in a KNO_3 medium the inhibition of pyruvate uptake by mitochondria increases as the triethyltin concentration is raised to 1 μM; further increase to 10 μM does not cause any additional inhibition. Since the depletion of substrate is presumably due to its displacement from the matrix by Cl^- (Harris *et al.*, 1973) the present data suggest that in the media used at least 1 μM-triethyltin is necessary to achieve equilibration of the Cl^- and OH^- gradients. This may be related to the lipid-solubility characteristics of triethyltin under these conditions. That no apparent 'carrier role' is exhibited in a KNO_3 medium is probably due to the lower lipid solubility of triethyltin nitrate or to its lack of formation.

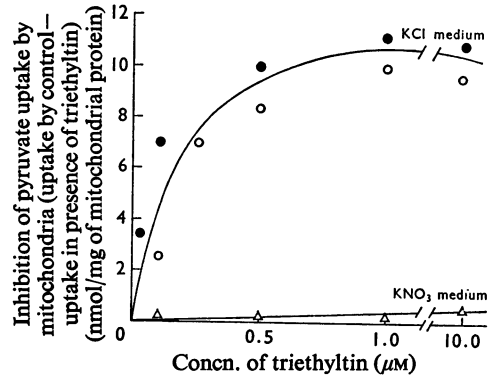

Fig. 1. *Effect of triethyltin concentration on pyruvate uptake by mitochondria*

The media used were those described in the Materials and Methods section in which the bulk constituent was either KCl or KNO_3 at 100 mM. The media contained 5 mM-[1-^{14}C]pyruvate (0.0667 μCi/μmol) in the presence of 1 mM-fumarate. Uptake of pyruvate by mitochondria was measured both enzymically (○, △) and radiochemically (●) as described in the Materials and Methods section. Points on the graph represent the mean of two determinations.

The data in Fig. 1 duplicate reasonably the observations of Aldridge & Street (1964) that for pyruvate in a KCl medium 0.1–1 μM-triethyltin caused an increase in the inhibition of oxidative phosphorylation of between about 25 and 80%, whereas very little inhibition occurred in a KNO_3 medium (Rose & Aldridge, 1972).

Effect of different trialkyltin and trialkyl-lead compounds on pyruvate uptake

In a KCl medium triethyltin, triethyl-lead, tributyltin and tributyl-lead at 1 μM all caused a similar decrease in pyruvate uptake by mitochondria in both the presence and the absence of rotenone. Trimethyltin and trimethyl-lead, however, were much less effective at 1 μM (Table 3) and the results reflect the relative effectiveness of the trialkyltin and trialkyl-lead compounds as inhibitors of oxidative phosphorylation observed previously (Aldridge *et al.*, 1962; Aldridge, 1958). In contrast, triethylgermanium, which is not an inhibitor of oxidative phosphorylation, was completely ineffective in causing displacement of pyruvate. This would be expected, since triethylgermanium does not mediate a Cl^-/OH^- ion exchange, probably because it exhibits a low ligand-exchange rate compared with the tin and lead analogues (Selwyn, 1972).

Table 3. *Effect of different trialkyltin and trialkyl-lead compounds on pyruvate uptake by mitochondria*

The medium used was that described in the Materials and Methods section in which the bulk constituent was KCl (100mM). Pyruvate was used at a concentration of 5mM in the presence of 1mM-fumarate, and where indicated rotenone (1.11 μM) and trialkylmetal compounds (1 μM) were added. Values for uptake are corrected for substrate present in the intermembrane space (see under 'Methods') and expressed as means ± S.E.M. from four determinations.

Trialkyl-compound present (1 μM)	Pyruvate uptake (nmol/mg of mitochondrial protein)	
	KCl	KCl+ rotenone
None	12.0±0.72*	21.6±0.66*
Triethyltin	3.3±0.14*	10.9±1.34*
Triethyl-lead	2.4±0.46	11.2±0.62
Tributyltin	1.2±0.34	9.6±0.54
Tributyl-lead	2.5±0.81	9.4±0.55
Trimethyltin	8.1±0.33	17.4±0.72
Trimethyl-lead	8.3±0.40	18.8±0.49
Triethylgermanium	13.1±0.21	20.3±0.26

* Values taken from Table 1.

Inhibition of respiration and ATP synthesis by triethyltin

Inhibition of respiration attributable to the Cl^-/OH^- exchange was originally demonstrated by Coleman & Palmer (1971). The results in Table 4 and 5 indicate that both mitochondrial O_2 uptake and ATP synthesis were sensitive to triethyltin in a KCl medium but not a KNO_3 medium. When pyruvate, malate or citrate were the oxidizable substrates state 3 respiration was inhibited 40–50% by triethyltin, but only a 16% inhibition was obtained when β-hydroxybutyrate was the substrate oxidized (Table 4). In this respect β-hydroxybutyrate behaved differently.

Previous studies have indicated that if triethyltin is added to mitochondria in a KCl medium in the absence of ADP a stimulation of O_2 uptake (uncoupling action) is observed (Aldridge, 1958; Stockdale *et al.*, 1970). Further, in the presence of ATP an adenosine triphosphatase activity about 30% of that caused by 30μM-2,4-dinitrophenol can be induced by 1μM-triethyltin (Aldridge & Street, 1964). Table 4 shows that the stimulation of O_2 uptake is Cl^- dependent and occurs under conditions when substrate uptake would be decreased (cf. Table 2). In this response β-hydroxybutyrate behaved similarly to the other substrates tested, which contrasts with its relative insensitivity to inhibition of state 3 respiration.

When ATP synthesis was measured by means of P_i uptake (Table 5) the inhibition by triethyltin was observed only in the KCl medium and was more

Table 4. *Inhibition of mitochondrial respiration by triethyltin*

The media used were those described in the Materials and Methods section and O_2 uptake was monitored polarographically. All substrates were present at 5mM and where indicated triethyltin was added at 1 μM and ADP (state 3) at 0.833mM. The resting respiration rates are those observed in the presence of substrate and ATP (3mM). Values are expressed as means ± S.E.M. from three determinations.

Respiration state	Respiration rate (ng-atoms of O/min per mg of mitochondrial protein)						
	KCl medium				KNO_3 medium		
	Pyruvate*	L-Malate	Citrate	DL-β-Hydroxybutyrate	Pyruvate	L-Malate	Citrate	DL-β-Hydroxybutyrate
Resting	19±3†	38±4	18±2	29±2	†19±2	34±3	21±2	33±4
Resting+triethyltin	46±6†	43±4	34±3	62±3	†24±4	34±3	22±2	39±5
State 3	125±14	50±3	98±5	147±8	136±12	44±2	104±9	157±13
State 3+triethyltin	66±8	26±1	58±2	123±7	127±10	41±3	103±11	140±12

* The medium also contained 1mM-fumarate (Aldridge & Street, 1971).
† Values similar to those observed by Rose & Aldridge (1972).

Table 5. *Inhibition of ATP synthesis by triethyltin*

The media used were those described in the Materials and Methods section and ATP synthesis was measured by means of P_i uptake. All substrates were present at 5mM; ADP was used at 3mM, and where indicated triethyltin at 1 μM. Values are expressed as means±S.E.M. from three determinations.

Medium	ATP synthesis (nmol of P_i uptake/min per mg of mitochondrial protein)			
	Pyruvate*	L-Malate	Citrate	DL-β-Hydroxybutyrate
KCl	262±18	122±13	132±15	363±27
KCl+triethyltin	103±7	11±5	14±6	247±20
KNO_3	255±24	104±9	134±10	324±24
KNO_3+triethyltin	249±30	104±11	136±13	312±21

* The medium also contained 1mM-fumarate (Aldridge & Street, 1971).

marked for all the substrates tested than for the corresponding inhibition of O_2 uptake (cf. Table 4). However, Table 5 indicates that the least effect was shown when β-hydroxybutyrate was the oxidizable substrate.

Discussion

The results of the present study show that 1 μM-triethyltin causes a marked decrease in the uptake of several NAD-linked substrates in a KCl but not in a KNO_3 medium. Trimethyltin, tributyltin and the trialkyl-lead analogues also impaired pyruvate uptake to various degrees by mitochondria in a KCl medium. The relative inhibition of the uptake of pyruvate caused by the different trialkyltin and trialkyl-lead compounds shows a good correlation with the relative effectiveness of these compounds at 1 μM in the inhibition of oxidative phosphorylation (Aldridge, 1958; Aldridge *et al.*, 1962).

However, when the concentration of either pyruvate, malate, citrate or β-hydroxybutyrate in the incubation medium was 5mM the concentrations of these substrates measured in the mitochondrial matrix in the presence of triethyltin were all estimated to be at millimolar concentrations, which should be adequate to facilitate maximum oxidation rates, e.g. the apparent K_m for the oxidation of pyruvate is estimated to be less than 1mM in heart (Bremmer, 1969) and may be as low as 0.05mM in liver (Linn *et al.*, 1969). The observation that under conditions when a decrease in substrate content occurs a stimulation of respiration is observed (Table 2, cf. Table 4) supports this conclusion.

Although the data in the present study concern substrate-uptake measurements made in the presence of ATP, other experiments have shown that under conditions of state 3 respiration in a KCl medium a similar decrease of substrate concentration is caused by 1 μM-triethyltin (D. N. Skilleter, unpublished work). The implication therefore is that the decrease of matrix substrate content is not the major explanation for the greater sensitivity of oxidative phosphorylation observed in KCl media by Rose & Aldridge (1972), particularly since these authors used substrate concentrations of 10mM in the media, which consequently would give rise to an even higher matrix concentration than that measured in the present study. This conclusion contrasts somewhat with the earlier studies by Manger (1969), which showed that the inhibition by triethyltin of substrate uptake could be partially overcome by increasing the external substrate concentration. This led him to conclude that substrate depletion might be an important factor in the inhibition of oxidative phosphorylation. However, the medium used was essentially sucrose in the presence of low KCl (5mM), and substrate concentrations used were less than 4mM. Therefore the data of Manger (1969) are not strictly comparable with the conditions used here. The simplest explanation of the data in the present study is that the primary event in KCl media is a triethyltin-mediated Cl^-/OH^- exchange, one consequence of which is a fall in the intramitochondrial pH. A similar situation would also be caused by halide ions other than Cl^-. This is probably the prime cause of the expulsion of intramitochondrial substrate (cf. McGivan & Klingenberg, 1971; Quagliariello *et al.*, 1971). The increase in the acidity of the matrix to equilibrate with that of the medium (in this case pH 6.8) has led Dawson & Selwyn (1974) to suggest that under these conditions the matrix enzymes operate at a lower rate. This could explain why in the present study ATP synthesis linked to the oxidation of pyruvate, malate and citrate was very sensitive to triethyltin, since their presence in the matrix is obligatory to their oxidation via the tricarboxylic acid cycle.

The results obtained in this study with β-hydroxybutyrate are particularly interesting. With regard to inhibition of its uptake into mitochondria by triethyltin it shows similar Cl^- sensitivity to uptakes of pyruvate, malate and citrate, indicating that it is taken up by the matrix and presumably displaced by Cl^- ions. Further, in the absence of ADP, respiration linked to the oxidation of β-hydroxybutyrate is stimulated by triethyltin, as is the oxidation of

pyruvate, malate and citrate, so that the parameters controlling this action of triethyltin probably apply to most substrates. However, the triethyltin inhibition of O_2 uptake and ATP synthesis linked to β-hydroxybutyrate oxidation under state 3 conditions shows relatively little Cl^- sensitivity compared with the other substrates tested. Since β-hydroxybutyrate dehydrogenase is located in the inner-membrane fraction of mitochondria (Werner & Neupert, 1972), inhibition of oxidative phosphorylation from β-hydroxybutyrate may be principally related to the halide-independent binding of triethyltin to its inhibitory site in the mitochondrial membrane. Unfortunately, it is not possible from the present data to determine if this Cl^- insensitivity is associated with the primary oxidation of β-hydroxybutyrate in the membrane or to its oxidation being associated with a distinct metabolic compartment, as postulated by Harris & Manger (1969). However, its further study could help lead to a greater understanding of the various actions of trialkyltin and trialkyl-lead compounds in what are evidently results of complicated interactions of several inter-related factors.

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