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Trialkyltins and Oxidative Phosphorylation

THE [^{32}P]PHOSPHATE–ADENOSINE TRIPHOSPHATE-EXCHANGE REACTION

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Both 2:4-dinitrophenol and trialkyltins influence the processes of oxidative phosphorylation in mitochondria. It is generally accepted that 2:4-dinitrophenol has a single action upon one of the steps of the energy-transferring chain between electron transport and the formation of adenosine triphosphate (for a full discussion and references see Aldridge & Parker, 1960; for definition of terms used to subdivide the processes of the respiratory chain, see Discussion). The stimulation of adenosine triphosphatase in mitochondria by 2:4-dinitrophenol is considered to be a consequence of the above-mentioned primary action. Trialkyltins inhibit both the oxygen uptake and adenosine triphosphatase stimulated by 2:4-dinitrophenol (Aldridge, 1958). Trialkyltins therefore act upon the energy-transferring chain which is involved in the action of 2:4-dinitrophenol (Aldridge, 1958; Aldridge & Parker, 1960).

The reversible exchange of orthophosphate with the phosphate of adenosine triphosphate is considered to be part of the energy-transferring chain in oxidative phosphorylation. This reaction has been demonstrated in mitochondria (Boyer, Falcone & Harrison, 1954; Boyer, Luchsinger & Falcone, 1956; Swanson, 1956; Löw, Siekevitz, Ernster & Lindberg, 1957) and in particles derived from mitochondria by various means but which still carry out oxidative phosphorylation (Cooper, Devlin & Lehninger, 1955; Cooper & Lehninger, 1957; Wadkins & Lehninger, 1957, 1959; Bronk & Kielley, 1957). 2:4-Dinitrophenol inhibits the exchange reaction at the same concentration as that which uncouples oxidative phosphorylation (Cooper & Lehninger, 1957; Boyer *et al.* 1956; Bronk & Kielley, 1958; Löw, Siekevitz, Ernster & Lindberg, 1958). In view of the association between the [^{32}P]phosphate–adenosine triphosphate-exchange reaction and oxidative phosphorylation and the inhibition by trialkyltins of some of the actions of 2:4-dinitrophenol, the influence of trialkyltins upon the exchange reaction has been examined.

METHODS

Abbreviations. The following abbreviations have been used throughout this paper: adenosine triphosphate (ATP) and adenosine diphosphate (ADP); radioactive phosphorus, ^{32}P; the exchange of ^{32}P (added as inorganic phosphate) with phosphorus atoms of ATP, ^{32}P–ATP-exchange reaction.

[^{32}P]*Phosphate-exchange reaction.* The medium used was basically that used for the assay of adenosine triphosphatase (Aldridge & Parker, 1960) but with an addition of inorganic phosphate to minimize the relative changes of inorganic phosphate concentration which might occur due to breakdown of ATP during the experiments. The rate of the exchange reaction was influenced by the phosphate concentrations, as shown in Fig. 1 (see also Cooper & Lehninger, 1957). For the standard test of ^{32}P exchange each beaker contained 3 ml. of a solution containing ATP (3 mM), KCl (10 mM), $MgCl_2$ (14 mM), ethylenediaminetetra-

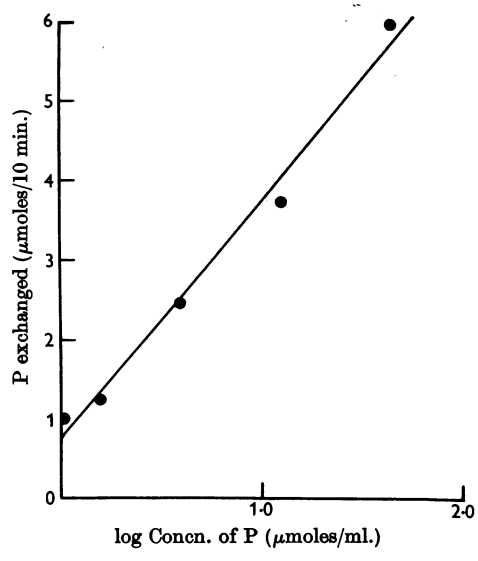

Fig. 1. Effect of phosphate concentration upon the ^{32}P–ATP exchange by mitochondria. Mitochondria (4·11 mg. of protein) were incubated for 10 min. at 37° in the medium used for the determination of adenosine triphosphatase (Aldridge & Parker, 1960) containing various amounts of inorganic phosphate. The concentration of phosphate plotted is the mean of the original and final determined concentrations. The liberation of phosphate during the incubation increased with increasing phosphate content of the medium and ranged from 0·4 to 1·8 μg.atoms of phosphorus. The μg.atom of phosphorus exchanged was calculated by the method of Boyer (1959).

acetic acid (1·0 mM), glycylglycine (16·7 mM), inorganic phosphate (3·3 mM), 1 μC of ^{32}P (equivalent to 200 000 recorded counts in the counting system used) and sucrose (30 mM). The beakers were shaken in air at 37°. After temperature equilibration 0·3 ml. of mitochondria suspension (equivalent to 150 mg. of original liver) was added and the mixture was incubated for 10 min. The reaction was stopped by the addition of 5 ml. of ice-cold 20% (w/v) perchloric acid. The precipitated protein was centrifuged off at 0° and the inorganic phosphate determined by the method of Fiske & Subbarow (1925). The concentrations and radioactivities of adenosine mono-, di- and triphosphates were determined as described by Stoner & Threlfall (1958) with 100 mg. of Norit charcoal and a sample of the perchloric acid extract of mitochondrial suspension equivalent to 60 mg. of original liver.

Under these conditions ATP and ADP were labelled with ^{32}P but the small amount of adenosine monophosphate contained a negligible amount of radioactivity. The results were calculated as counts/μg.atom of exchangeable phosphorus on the basis that the β- and γ-phosphorus atoms of ATP and the β-phosphorus atom of ADP were labelled. The incorporation of ^{32}P into the β-phosphorus atom of ADP is presumably due to myokinase present in rat-liver mitochondria (Barkulis & Lehninger, 1951). Particles derived from rat-liver mitochondria by treatment with digitonin do not contain myokinase activity and during the exchange reaction only the γ-phosphorus atom of ATP is labelled, whereas ADP is unlabelled (Cooper & Lehninger, 1957). In our experiments the activity of the β-phosphorus atom of ADP was always 60–80% of the mean activity of the β- and γ-phosphorus atoms of ATP.

Preparation of the mitochondria. Rat-liver mitochondria were prepared as previously described (Aldridge, 1957, 1958) with a Potter–Elvehjem-type homogenizer with a smooth glass tube and Perspex pestle with a total clearance of 0·02 in. The construction of the homogenizer has been described (Aldridge, Emery & Street, 1960).

Inhibitors. Triethyltin and tri-*n*-butyltin acetates were supplied by Dr G. J. M. Van der Kerk. These compounds were synthesized by published methods (Van der Kerk & Luijten, 1956). Dimethylformamide is an excellent solvent for trialkyltins, allowing stock solutions (usually 0·02 M) to be prepared. A sample (0·03 ml.) of these solutions was added to each beaker, final concentrations of 1% (v/v) dimethylformamide never being exceeded. This concentration has a negligible effect upon the activity of the control mitochondria.

Special chemicals. The following chemicals were obtained from the sources indicated: glycylglycine (Roche Products Ltd.), disodium salt of ATP (Sigma Chemical Co., St Louis, Mo., U.S.A.) and radioactive phosphate (^{32}P, 1 mC/10 ml. in a solution of sterile iso-osmotic phosphate buffer, pH 7, containing 1 mg. of P/ml., The Radiochemical Centre, Amersham, Bucks.).

Analytical methods. Protein was measured by the biuret method of Robinson & Hogden (1940) as modified by Aldridge (1957). Mitochondrial protein has been expressed as milligrams of albumin. Triethyltin and tri-*n*-butyltin were determined by the method of Aldridge & Cremer (1957) as modified by Cremer (1957).

Where sufficient determinations have been made the results are expressed as mean ± S.E. with the number of observations in parentheses.

RESULTS

Phosphate-exchange reaction

A range of concentrations of trialkyltins were used in the medium for the assay of the exchange reaction. The results given in Table 1 show that triethyltin prevents this reaction, a concentration of 0·4 μM causing 50% inhibition. Similar experiments with tri-*n*-butyltin (Table 2) showed that a concentration of 0·65 μM produced 50% inhibition.

Table 1. *Inhibition by triethyltin of the* [^{32}P]*phosphate–adenosine triphosphate exchange of liver mitochondria*

For details of techniques used see Methods section. Each set of figures represents a different preparation of mitochondria. The results are calculated upon the basis that ATP contains 2 exchangeable phosphorus atoms. 'Equilibrium' is the calculated specific activity at equilibrium of the inorganic and β- and γ-phosphorus atoms of ATP. On this basis the specific activity of the β-phosphorus atom of ADP was 60–80% of the mean activity of the β- and γ-phosphorus atoms of ATP. The concentration of triethyltin inhibiting the exchange by 50% (determined graphically) is 0·4 μM.

Concn. of triethyltin (μM)	Specific activity of ATP (counts/μmole of exchangeable P)			Mean (% of control)
	Expt. 1	Expt. 2	Expt. 3	
Nil	3080	2940	2850	100
2·5	—	320	477	14
1·25	678	555	695	22
0·625	—	895	1190	36
0·312	1880	1580	1825	63
0·156	—	2110	2270	76
0·078	2630	—	—	86
Equilibrium	7530	8000	6130	—

Table 2. *Inhibition by tri*-n-*butyltin of the* [^{32}P]*phosphate–adenosine triphosphate exchange of liver mitochondria*

For experimental conditions see Methods section and Table 1. The specific activity of the β-phosphorus atom of ADP was 80–90% of the mean activity of the β- and γ-phosphorus atoms of ATP. 'Equilibrium' is defined as in Table 1. The concentration of tri-*n*-butyltin inhibiting the exchange by 50% (determined graphically) is 0·65 μM.

Concn. of tri-*n*-butyltin (μM)	Specific activity of ATP (counts/μmole of exchangeable P)		Mean (% of control)
	Expt. 1	Expt. 2	
Nil	2580	3630	100
12·5	112	—	4
3·22	158	334	7
1·66	—	343	9
0·805	436	1050	23
0·415	—	2340	64
0·201	2050	3320	85
Equilibrium	6680	7350	—

In these experiments the medium contained 3–4 mM-inorganic phosphate and the gas phase was air. However, in a few preliminary experiments before the conditions were standardized, triethyltin and tri-*n*-butyltin were shown to inhibit the exchange reaction in the absence of added inorganic phosphate and with nitrogen as the gas phase.

Trialkyltins inhibit many other reactions associated with oxidative phosphorylation (Aldridge & Cremer, 1955; Aldridge, 1958). In Fig. 2 the effect of a range of concentrations of tri-*n*-butyltin upon six of these processes is shown. The results for each process are taken from a single experiment and in each experiment a different preparation of mitochondria was used. In Fig. 2 there is considerable scatter of the results around the line drawn through the mean values. In our experience this is the kind of reproducibility to expect from such experiments and particularly so with a steep inhibition curve. We therefore consider that the relationship between the concentration of tri-*n*-butyltin and the observed inhibition is identical for the different processes. The concentration of tri-*n*-butyltin inhibiting all of these processes by 50 % falls within the range 0·6–1·0 μM and is for triethyltin between 0·2 and 0·4 μM.

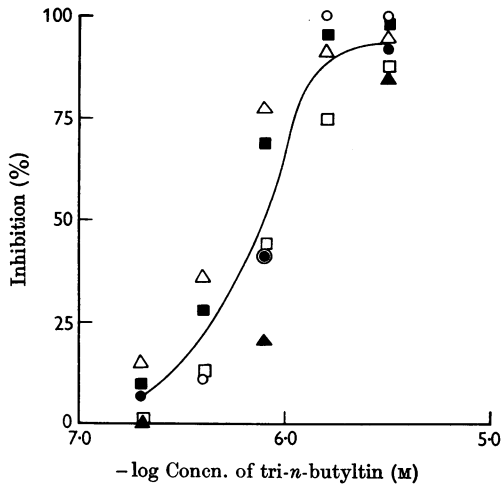

Fig. 2. Inhibition by tri-*n*-butyltin of various activities associated with the respiratory chain. With the exception of the ^{32}P–ATP exchange, the various activities were determined as previously described (Aldridge, 1958). Pyruvate + fumarate was the substrate in all the experiments involving O_2 uptake. ○, Adenosine triphosphatase activated by 2:4-dinitrophenol; ●, O_2 uptake induced by potato apyrase; △, ^{32}P–ATP-exchange reaction; ▲, O_2 uptake induced by 2:4-dinitrophenol; □, O_2 uptake in the presence of excess of hexokinase and glucose; ■, P uptake in the presence of excess of hexokinase and glucose. The line plotted passes through the means of the values obtained at each of the concentrations studied.

Distribution of triethyl- and tri-n-butyl-tin between mitochondria and aqueous medium

Oxidative phosphorylation can be carried out by particles derived from rat-liver mitochondria by treatment with digitonin (Cooper *et al.* 1955). A. L. Lehninger & C. L. Wadkins (unpublished observations; see Discussion) have shown that triethyltin and tri-*n*-butyltin inhibit oxidative phosphorylation carried out by these particles but the concentrations required were higher than those necessary with intact mitochondria. This suggested that intact mitochondria were able to concentrate these compounds and disrupted mitochondria were not. The results in Table 3 show that

Table 3. *Distribution of triethyltin between mitochondria and suspending medium*

Mitochondrial suspension (4 ml.) in 0·3 M-sucrose [equivalent to 2 g. wet wt. of liver and containing 51·8 ± 2·1 (6) mg. of protein] was added to 4 ml. of medium containing triethyltin. The mixture was kept ice-cold for 5 min. and then centrifuged at 12 000 *g* for 20 min. Triethyltin was determined in both the mitochondrial pellet and supernatant. The percentage recovery of added triethyltin was 95·6 ± 1·9 (17). The distribution is the quotient [μg. of triethyltin/g. wet wt. of mitochondria divided by the concentration (μg./ml.) in the supernatant]. The wet wt. of mitochondria was calculated from the protein content and the results of Werkheiser & Bartley (1957).

Medium	Overall concn. of triethyltin (μM)	Distribution (mitochondria/medium)
0·3 M-Sucrose	40	67·9 ± 4·9 (4)
Water	40	66 (1)
0·15 M-KCl	40	30·4 ± 1·4 (8)
0·15 M-NaCl	40	31 (1)
Medium for adenosine-triphosphatase assay	40	24 (1)
Medium for ^{32}P–ATP-exchange reaction	17	25·3 (1)
	25	27·5 (1)
	33	25·2 (1)

Table 4. *Distribution of tri-n-butyltin between mitochondria and medium*

Conditions were as described in Table 3. Tri-*n*-butyltin could be determined in the supernatant [percentage recovery 99·0 ± 1·8 (7)] but recovery from the mitochondrial pellet was never better than 50 %. The solubility of tri-*n*-butyltin in 0·15 M-KCl at 0° is 0·36 mM.

Medium	Concn. of tri-*n*-butyltin added (μM)	Concn. of tri-*n*-butyltin in supernatant (μM)
0·15 M-KCl	14	0
	21	0
	29	1·1*
Medium for ^{32}P–ATP-exchange reaction	14	0
	21	1·1*
	29	2·0*

* These concentrations are near the limit of the method.

the concentration of triethyltin in mitochondria is 60–70 times that in the aqueous suspending medium when the medium is 0·3 M-sucrose and 25–30 times when the mitochondria are suspended in an ionic medium. Whether the cation is sodium or potassium does not appear to influence the distribution. Triethyltin could be readily determined in both the mitochondrial pellet and the suspending medium. Tri-*n*-butyltin could be determined in the supernatant but we were never able to recover more than 50 % from the mitochondria. Determinations of tri-*n*-butyltin remaining in the suspending medium indicate that most of it is removed from the medium and presumably adsorbed on or concentrated in the mitochondria (Table 4).

DISCUSSION

For the purpose of this paper the following definitions have been used. The respiratory chain is regarded as a physiological entity concerned with all of the processes involved in the oxidation of substrates and the associated oxidative phosphorylation. The electron-transport chain is the structure in which occur the processes involving the transfer of electrons from coenzyme I through the flavin and cytochromes to oxygen. The energy-transferring chains are concerned with the processes whereby the energy of oxidation of electron-transport intermediates is used for the synthesis from inorganic phosphate of the terminal pyrophosphate bonds of ATP.

Triethyltin and tri-*n*-butyltin are potent inhibitors of the ^{32}P–ATP-exchange reaction in rat-liver mitochondria. When the respiratory carriers are maintained in a reduced state the ^{32}P–ATP exchange is inhibited (Wadkins & Lehninger, 1957; 1959) and it may be argued that since the trialkyltins prevent respiration this is the explanation of their effect upon the ^{32}P–ATP exchange. However, the conditions necessary to produce such inhibition must be carefully controlled [high concentrations of cyanide, or nitrogen as the gas phase, and an oxidizable substrate such as β-hydroxybutyrate must be present (Wadkins & Lehninger, 1957; 1959)]. It is clear, however, that without substrate and with intact mitochondria concentrations of respiratory inhibitors such as cyanide, antimycin A and amytal sufficient for complete inhibition of respiration produce less than 50 % inhibition of the ^{32}P–ATP exchange (Jalling, Löw, Ernster & Lindberg, 1957; Löw *et al.* 1958; Boyer *et al.* 1956). The experiments with trialkyltins were carried out in the absence of added substrate and in air and there is close agreement between the inhibition of oxygen uptake and of the ^{32}P–ATP exchange (Fig. 2). The effect of trialkyltins upon the ^{32}P–ATP exchange cannot therefore be explained on the basis of an effect due to its inhibition of respiration.

The concentrations which are effective may be regarded as identical with those which inhibit other activities associated with the energy-transferring processes of the respiratory chain (Fig. 2). The inhibitory action of the trialkyltins upon the exchange reaction is in agreement with the widely held view that this reaction is associated with oxidative phosphorylation (Cooper & Lehninger, 1957; Boyer *et al.* 1956; Löw *et al.* 1958). The slope of the curve relating inhibition of the ^{32}P–ATP exchange to the concentration of tri-*n*-butyltin is very similar to and is probably identical with that for other processes associated with oxidative phosphorylation. The concentration of tri-*n*-butyltin (3·0 μM) producing almost complete inhibition of the exchange reaction also produces almost identical inhibition of the other processes. Therefore all of the ^{32}P–ATP exchange in rat-liver mitochondria is associated with the processes of oxidative phosphorylation.

A. L. Lehninger & C. L. Wadkins (unpublished observations) have shown that triethyltin and tri-*n*-butyltin affect oxidative phosphorylation and the ^{32}P–ATP-exchange reaction in particles derived from mitochondria by treatment with digitonin (Devlin & Lehninger, 1958). Both activities were inhibited by 1–10 μM-triethyltin and by 0·01–0·1 mM-tri-*n*-butyltin. The ADP–ATP exchange was not inhibited by these concentrations. The evidence for the association of the latter reaction with oxidative phosphorylation is not so strong as for the ^{32}P–ATP exchange. However, it is difficult to visualize a mechanism of ^{32}P–ATP exchange that would not involve an ADP–ATP exchange also. In addition the sensitivity of the ADP–ATP exchange in fresh preparations of submitochondrial particles to 2:4-dinitrophenol, dicoumarol and gramicidin is strong evidence for its association with oxidative phosphorylation (Wadkins & Lehninger, 1958). Trialkyltins do not inhibit the ADP–ATP exchange and in this respect they resemble azide (Wadkins & Lehninger, 1958; Lehninger, Wadkins & Remmart, 1959).

Trialkyltins inhibit the activities of intact and disrupted mitochondria associated with oxidative phosphorylation. This indicates that the whole structure of mitochondria is not required for the action of trialkyltins. However, the concentrations of trialkyltins required to inhibit oxidative phosphorylation in submitochondrial particles are higher than those for intact mitochondria. This difference is probably explained by the higher concentration of triethyltin and tri-*n*-butyltin found in intact mitochondria than in the surrounding medium. Intact mitochondria therefore concentrate trialkyltins and it is possible that the sub-

mitochondrial particles do not possess this property. This distribution of trialkyltins between intact mitochondria and the medium may modify our views on the intrinsic activity of each trialkyltin against the oxidative-phosphorylation system of mitochondria. The physical properties, and particularly solubility in lipid, vary appreciably in the different homologues and it is probable that each homologue will be differently distributed. In fact a further examination of the relation between their solubility in lipid and their concentration by intact mitochondria may provide evidence whether it is trialkyltin adsorbed upon the lipoprotein structure of the mitochondria which is active against oxidative phosphorylation.

2:4-Dinitrophenol stimulates adenosine triphosphatase in mitochondria and trialkyltins prevent this stimulation. Both 2:4-dinitrophenol (Cooper & Lehninger, 1957; Boyer *et al.* 1956; Bronk & Kielley, 1958; Löw *et al.* 1958) and trialkyltins inhibit the ^{32}P–ATP-exchange reaction. We considered that these relationships might enable us to decide whether or not 2:4-dinitrophenol was acting upon a phosphorylated intermediate. Schemes similar to that of Fig. 3 have been used by many workers in this field (Lehninger, 1953; Slater, 1953; Chance & Williams, 1956; Lardy, 1955). Slater's scheme (Slater, 1953; Myers & Slater, 1957) has been regarded as representative but an extra intermediate (Y) has been added to take account of the following considerations.

(*a*) Since the respiratory carriers associated with the three steps in electron transport associated with ATP formation are very different chemically it is unlikely that they will react with the same intermediate (I) and therefore three different intermediates will be involved.

$$(1)\quad AH_2+B+I \rightleftharpoons A\sim I+BH_2$$

$$(2)\quad A\sim I+X \rightleftharpoons X\sim I+A$$

$$(3)\quad X\sim I+Y \rightleftharpoons X\sim Y+I$$

$$(4)\quad X\sim Y+P_i \rightleftharpoons Y\sim P+X$$

$$(5)\quad Y\sim P+ADP \rightleftharpoons ATP+Y$$

Fig. 3. Hypothetical scheme for the energy-transferring chain in oxidative phosphorylation. The scheme is basically that of Slater (Slater, 1953; Myers & Slater, 1957), with an additional hypothetical intermediate Y to take account of views (Aldridge & Parker, 1960) that 2:4-dinitrophenol is acting upon an intermediate common to all of the three energy-transferring chains associated with electron transport (see Discussion). A and B represent coenzymes or respiratory pigments of the electron-transport chain.

(*b*) The three energy-transferring chains associated with electron transport have a similar sensitivity to 2:4-dinitrophenol and are therefore considered to consist of identical reactions between the point of action of 2:4-dinitrophenol and the reaction forming ATP (Aldridge & Parker, 1960).

(*c*) The ADP–ATP exchange is not itself sensitive to 2:4-dinitrophenol (Wadkins & Lehninger, 1958).

This scheme can now be used for discussion of the mode of action of 2:4-dinitrophenol and the trialkyltins. If it is assumed that all the reactions (1–5) are mediated by different enzymes (indicated by the arrows) then trialkyltins could exert their effects in two ways: by reacting either with an intermediate or with an enzyme. If an enzyme is inhibited, then, since trialkyltins inhibit at the same concentration both uptake of oxygen and adenosine triphosphatase stimulated by 2:4-dinitrophenol, the same enzyme must be involved in the action of these two substances (Aldridge, 1958). This enzyme cannot be that for reaction 5, the ADP–ATP exchange, since it is insensitive to trialkyltins. Since trialkyltins inhibit at the same concentration the adenosine triphosphatase stimulated by 2:4-dinitrophenol and the ^{32}P–ATP exchange, it seems that 2:4-dinitrophenol is reacting at some stage after the incorporation of phosphate (even if this be an enzyme complex involving phosphorus).

If trialkyltins are reacting with an intermediate to form an undissociated complex it would have to be with X to take account of the following. (*a*) As previously pointed out it is likely that three different intermediates (I_1, I_2, I_3) would be involved in the three energy-transferring chains associated with electron transport. Trialkyltins inhibit at least two of these chains (Aldridge & Cremer, 1955; Aldridge, 1958; Aldridge & Parker, 1960) and it is assumed that trialkyltins will react with an intermediate common to all three steps (therefore not I). (*b*) The ADP–ATP exchange is not affected by trialkyltin, so eliminating Y.

If trialkyltins react with X to form an undissociated R_3Sn–X, then reaction (2) will be prevented in the forward direction and reaction (4) in the reverse direction. 2:4-Dinitrophenol can therefore react only with X $\sim$ Y; reaction with Y $\sim$ P is excluded by the fact that the reaction (5), the ADP–ATP exchange, is not inhibited by trialkyltins. It is apparent from the same scheme that it is not possible to conclude whether 2:4-dinitrophenol reacts with a phosphorylated or a non-phosphorus-containing intermediate. Experiments have been carried out (Borst & Slater, 1959; 1960) to examine the requirement for phosphate of the stimulation by 2:4-dinitrophenol of the uptake of oxygen of rat-liver mitochondria with various substrates. It

was concluded that phosphate was not required for this stimulation and that 2:4-dinitrophenol reacts with an energy-rich intermediate in oxidative phosphorylation formed before the intervention of inorganic phosphate (Borst & Slater, 1960). The details of the relevant experiments have not yet been published but the report does not seem to eliminate the possibility that a small amount of inorganic phosphate was within the mitochondria and was 'turning over' rapidly. We therefore consider that the question of the involvement of phosphorus in the mechanism of action of 2:4-dinitrophenol is still not conclusively answered.

SUMMARY

1. Triethyltin and tri-*n*-butyltin inhibit the [^{32}P]phosphate–adenosine triphosphate-exchange reaction in rat-liver mitochondria. The exchange reaction and the other reactions associated with oxidative phosphorylation are all inhibited at the same concentration.

2. After incubation of rat-liver mitochondria in a medium containing trialkyltin, the concentration of trialkyltin is much higher in the mitochondria than in the medium.

3. These findings are discussed in relation to the mechanism of action of trialkyltins and 2:4-dinitrophenol upon the processes of oxidative phosphorylation.

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REFERENCES

Aldridge, W. N. (1957). *Biochem. J.* **67**, 423.
Aldridge, W. N. (1958). *Biochem. J.* **69**, 367.
Aldridge, W. N. & Cremer, J. E. (1955). *Biochem. J.* **61**, 406.
Aldridge, W. N. & Cremer, J. E. (1957). *Analyst*, **82**, 37.
Aldridge, W. N., Emery, R. C. & Street, B. W. (1960). *Biochem. J.* **77**, 326.
Aldridge, W. N. & Parker, V. P. (1960). *Biochem. J.* **76**, 47.
Barkulis, S. S. & Lehninger, A. L. (1951). *J. biol. Chem.* **190**, 339.
Borst, P. & Slater, E. C. (1959). *Nature, Lond.*, **184**, 1396.
Borst, P. & Slater, E. C. (1960). *Nature, Lond.*, **185**, 537.
Boyer, P. D. (1959). *Arch. Biochem. Biophys.* **82**, 387.
Boyer, P. D., Falcone, A. B. & Harrison, W. H. (1954). *Nature, Lond.*, **174**, 401.
Boyer, P. D., Luchsinger, W. W. & Falcone, A. B. (1956). *J. biol. Chem.* **223**, 405.
Bronk, J. R. & Kielley, W. W. (1957). *Biochim. biophys. Acta*, **24**, 440.
Bronk, J. R. & Kielley, W. W. (1958). *Biochim. biophys. Acta*, **29**, 369.
Chance, B. & Williams, G. R. (1956). *Advanc. Enzymol.* **17**, 65.
Cooper, C., Devlin, T. M. & Lehninger, A. L. (1955). *Biochim. biophys. Acta*, **18**, 159.
Cooper, C. & Lehninger, A. L. (1957). *J. biol. Chem.* **224**, 561.
Cremer, J. E. (1957). *Biochem. J.* **67**, 87.
Devlin, T. M. & Lehninger, A. L. (1958). *J. biol. Chem.* **233**, 1586.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Jalling, O., Löw, H., Ernster, L. & Lindberg, O. (1957). *Biochim. biophys. Acta*, **26**, 231.
Lardy, H. A. (1955). *Proc. 3rd int. Congr. Biochem., Brussels*, p. 287.
Lehninger, A. L. (1953). *Harvey Lect.* **49**, 176.
Lehninger, A. L., Wadkins, C. L. & Remmart, L. F. (1959). *Ciba Foundation Symp., Regulation of Cell Metabolism*, p. 130.
Löw, H., Siekevitz, P., Ernster, L. & Lindberg, O. (1957). *Biochim. biophys. Acta*, **26**, 224.
Löw, H., Siekevitz, P., Ernster, L. & Lindberg, O. (1958). *Biochim. biophys. Acta*, **29**, 392.
Myers, D. K. & Slater, E. C. (1957). *Biochem. J.* **67**, 572.
Robinson, H. W. & Hogden, C. G. (1940). *J. biol. Chem.* **135**, 709.
Slater, E. C. (1953). *Nature, Lond.*, **172**, 975.
Stoner, H. B. & Threlfall, C. J. (1958). *Biochem. J.* **69**, 376.
Swanson, M. A. (1956). *Biochim. biophys. Acta*, **20**, 85.
Van der Kerk, G. J. M. & Luijten, J. G. A. (1956). *J. appl. Chem.* **6**, 49.
Wadkins, C. L. & Lehninger, A. L. (1957). *J. Amer. chem. Soc.* **79**, 1010.
Wadkins, C. L. & Lehninger, A. L. (1958). *J. biol. Chem.* **233**, 1589.
Wadkins, C. L. & Lehninger, A. L. (1959). *J. biol. Chem.* **234**, 681.
Werkheiser, W. C. & Bartley, W. (1957). *Biochem. J.* **66**, 79.