Toxicity of Organotin Compounds

IV. Impairment of Energy Metabolism of Rat Thymocytes by Various Dialkyltin Compounds

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Toxicity of Organotin Compounds. IV. Impairment of Energy Metabolism of Rat Thymocytes by Various Dialkyltin Compounds. PENNINKS, A. H., AND SEINEN, W. (1980). *Toxicol. Appl. Pharmacol.* **56**, 221–231. Isolated thymocytes were incubated with various carbohydrates in the presence of dimethyltindichloride (DMTC), diethyltindichloride (DETC), di-*n*-butyltindichloride (DBTC), and di-*n*-octyltindichloride (DOTC) and the substrate conversion, oxygen consumption, and cell viability were measured. All the dialkyltin compounds produced a dose-dependent stimulation of the glucose consumption, but at different concentrations. The most toxic homologs, DBTC and DETC, stimulated the glucose consumption maximally at levels of 5 and 10 μM, respectively, whereas DMTC and DOTC were less active, inducing a maximum stimulation at 120 μM. At higher exposure levels a sharp fall in the stimulation of the glucose consumption was noted together with a decrease of the oxygen consumption and viability of the thymocytes. The oxidative metabolism of glucose, lactate, and pyruvate by the TCA cycle was inhibited by dialkyltin compounds in a dose-related fashion. The glycolytic pathway was not affected, since under anaerobic conditions the glucose consumption was similar to that of the controls up to levels inducing a maximum stimulation of the glucose consumption under aerobic conditions. Probably the entrance of the glycolytic end products into the TCA cyle is disturbed by an inhibition of the pyruvate dehydrogenase system. Additional support for an interference with α-keto acid dehydrogenase complexes were obtained from mitochondrial incubations. DBTC inhibited the pyruvate and α-ketoglutarate conversion, oxygen consumption and ATP production of rat liver mitochondria in a dose-related fashion. Although *in vitro* the dialkyltin homologs display a comparable mode of action on the energy metabolism of rat thymocytes, *in vivo* especially DBTC and DOTC induce thymus involution whereas DMTC and DETC hardly decrease thymus weight. This discrepancy may be related with a different distribution pattern of the water-soluble lower homologs and the fat-soluble higher homologs. Therefore, we conclude that the inhibition of the energy metabolism of rat thymocytes by dialkyltins is related to their thymolytic effects *in vivo*.

Dialkyltin compounds are primarily used in industry, in particular they are used as heat stabilizers of polyvinyl chloride (Ross, 1965; Luyten, 1972; van der Kerk, 1975, 1978). For food packing purpose some dioctyltin derivatives are permitted by the U.S. Food and Drug Administration. In European countries dimethyltins are also allowed as additives in polyvinyl chloride products which come in contact with food.

The toxicity of organotin compounds has been reviewed by Barnes and Stoner (1959) and by Piver (1973). No attention was paid to the effects of dialkyltin compounds on the

lymphoid system. However, Seinen and co-authors have shown that for some organotin compounds, especially di-n-butyltin (DBTC) and di-n-octyltindichloride (DOTC) the effects on the immune system are the most sensitive parameters of toxicity (for review see Seinen and Penninks, 1979). Thymus atrophy was the most prominent feature of dialkyltin toxicity (Seinen and Willems, 1976). Thymus weights of rats fed 50 or 150 ppm DOTC for 4 weeks were only 48 and 16% of the control weights. Histologically lymphocyte depletion was observed in thymus and thymus-dependent areas of spleen and lymphnodes, whereas no treatment-related histopathological changes were noted in other organs. Comparative studies with various dialkyltin compounds (Seinen et al., 1977) revealed that not only DOTC, but also DBTC and to a lesser extent di-n-propyltindichloride (DPTC) and diethyltindichloride (DETC) reduced the weights of thymus, spleen, and popliteal lymphnodes of rats in a dose-related fashion both upon oral and intravenous application. In contrast, dimethyltindichloride (DMTC) did not cause lymphoid atrophy. The reduction of lymphoid organ weights was caused by a progressive fall in the number and viability of lymphocytes that could be isolated from these organs. In vitro, the dialkyltin compounds were extremely cytotoxic. Concentrations as low as 0.1 μg DBTC/ml medium completely inhibited the proliferation of rat and human thymocytes as measured by the incorporation of [^{3}H]thymidine into DNA (Seinen et al., 1979).

DBTC and DOTC exert a cytotoxic action upon thymic lymphocytes, but, in contrast to other cytotoxic agents, they do not induce a generalized cytotoxicity nor a myelotoxicity (Seinen and Penninks, 1979). The number and viability of bone marrow cells nor the number of bone marrow stem cells, as measured by the spleen colony assay (Till and McCulloch, 1961) was reduced. As a consequence of their selective cytotoxic effect upon thymus lymphocytes DBTC and

DOTC induce immunosuppression, especially in those test systems in which T lymphocytes are predominantly involved (Seinen and Willems, 1976; Seinen et al., 1979; Seinen, 1980).

The mechanism of the selective cytotoxicity of dialkyltin compounds is unknown. Aldridge and Cremer (1955) have studied the effects of DETC on metabolic processes in rat brain brei and rat liver mitochondria. These studies indicated that the oxidative phosphorylation was reduced by DETC due to an inhibition of pyruvate- and α-ketoglutarate dehydrogenase complexes. Comparable effects on rat liver mitochondria were observed with other dialkyltin homologs from methyl to hexyl (Aldridge, 1976). Besides these effects on the α-keto acid dehydrogenase systems, Cain et al. (1977) has reported that DBTC inhibited mitochondrial oxidative phosphorylation, similar to the classical inhibitor olygomycin. Effects of dialkyltin compounds on the plasma membrane of thymocytes were suggested by Miller (1978), since he observed an increase of sulfydryl groups on the lymphocytes associated with an inhibited amino acid transport.

In view of the selective effects of dialkyltins on thymus, the present study deals with the effects of these compounds on the metabolism of rat thymocytes. Various homologs are compared, since the in vivo-induced thymus involution is highly dependent on the length of the alkyltin chain. The energy metabolism of thymocytes is disturbed by all of the alkyltin compounds tested, but at different concentrations.

METHODS

Materials. Di-n-octyltindichloride (DOTC), di-n-butyltindichloride (DBTC), diethyltindichloride (DETC), and dimethyltindichloride (DMTC) all with a purity of more than 99% were kindly provided by Dr. E. J. Bulten.[1]

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[1] Obtained from Dr. E. J. Bulten, Institute for Organic Chemistry, TNO, Utrecht, The Netherlands.

Animals. Specific pathogen-free male Wistar-derived (WU-CPB) rats[2] of 125–150 g were used for the preparation of thymic lymphocytes and liver mitochondria.

Cell preparations. Immediately after decapitation of the rats, thymus glands were removed and placed in cold phosphate-buffered saline (PBS) for the preparation of rat lymphocyte suspensions. The thymuses were minced with scissors in fragments which were gently pressed through a nylon sieve with a pore diameter of 220 μm. To remove cell clumps, the suspensions were drawn through a 25-gauge needle, and the cells were washed twice with PBS. All handlings were carried out in the cold.

Cells were counted with an electronic particle counter[3] and their viability was determined with a dye exclusion test (0.05% nigrosine[4] solution in saline). At the start of the experiments cell viability was always more than 95%.

Cell incubation. For the metabolism studies 2 ml of a suspension of 5×10^7 thymocytes/ml PBS was incubated in 15-ml Warburg vessels of a respirometer[5] with various carbohydrates for 4 hr at 37°C. The side arm of the vessels contained 1 ml of the various substrates[6] dissolved in PBS adjusted to pH 7.5. After a 10-min preincubation for temperature equilibration the substrates were added to the cells. If not otherwise noted the final substrate concentration was 2 mM. In the center well of the vessels 200 μl of a 20% potassium hydroxide solution and filter paper was present for carbon dioxide trapping.

The organotin compounds (always freshly prepared) were dissolved in ethanol at various concentrations and added to the vessels just before the start of the incubations. The final ethanol concentration was always 0.5%, a concentration that did not effect the test system.

Oxygen consumption was measured with air as gas phase during the whole incubation period and expressed in micromoles per 10^8 cells per 4 hr. When anaerobiosis was required the PBS as well as the vessels were gassed with pure nitrogen.

Both, at the start and at the end of the incubation period, a sample of the cell suspensions was mixed with an equal volume of ice-cold 0.66 M perchloric acid. After 15 min in the cold the mixture was centrifuged at 400g. In the neutralized supernatant glucose,

lactate, and pyruvate concentrations were measured by standard enzymatic procedures[7] (Bergmeyer, 1974). The mean consumption or production of the respective compounds in triplicate incubations was calculated from the concentrations before and after the experiments. All results are expressed in micromoles per 10^7 cells per 4 hr and are the mean of at least three experiments.

The number and viability of the thymocytes were checked immediately after the end of the incubation.

Preparation of mitochondria. Mitochondria were prepared from rat liver according to Meyers and Slater (1957). Briefly, livers were removed and placed in ice-cold medium containing 0.25 M mannitol, 5 mM Tris, and 2 mM EDTA-Na$_2$ (pH 7.5). Mannitol was used instead of sucrose since sucrose will interfere with glucose determinations. After mincing with scissors the fine liver fragments were washed several times and homogenized with a Potter–Elvejhem. The homogenate was centrifuged twice during 5 min at 755g to remove nuclei, erythrocytes, intact liver cells and debris. Centrifugation of the supernatant at 4500g for 10 min gave a mitochondrial pellet. This fraction was washed once by resuspension and centrifugation for 10 min at 12600g. Finally, the mitochondria were suspended at a protein concentration of 13.5 mg/ml. All centrifugations were carried out at 0–5°C. Protein content was measured according to Lowry (1951).

Incubation of mitochondria. The rat liver mitochondria were incubated in 15-ml Warburg respirometer vessels for 40 min at 25°C and vigorously shaken. Each vessel contained 2 ml of a standard reaction medium of 25 mM mannitol, 15 mM KCl, 2 mM EDTA, 5 mM MgCl$_2$, 50 mM Tris/HCl (pH 7.5), and 5 mM potassium phosphate (pH 7.5) supplemented with 1 mM ADP, 30 mM glucose, and 2.5 U hexokinase per milliliter. Glucose and hexokinase were added to insure that the ADP concentration could not become the limiting factor in the 40 min incubations. To each vessel 0.5 ml of the mitochondrial suspension was added followed by the addition of various concentrations of DBTC in ethanol (final conc. 0.5%). After a 10-min preincubation the reactions were started by the addition of 0.5 ml substrate solution (final concentration 5 mM pyruvate and 5 mM malate or 5 mM α-ketoglutarate and 5 mM malate) from the side arm of the vessels and followed during 30 min.

Carbon dioxide was trapped by 200 μl of a 20% potassium hydroxide solution and filter paper in the center well of each vessel. Oxygen consumption was measured with air as gas phase during the entire incubation period and expressed in micromoles per milligram of mitochondrial protein per 30 min.

At the start and at the end of the incubation period

TABLE 1

EFFECT OF VARIOUS SUBSTRATES ON THE ENDOG-
ENOUS RESPIRATION OF ISOLATED RAT THYMUS CELLS[a]

Substrate	Concen- tration (mM)	Oxygen consumption (μmol/10^8 cells/4 hr)	Percentage of control
None	—	2.86 ± 0.04 (3)[b]	
Glucose	2	4.16 ± 0.09 (10)	145
Pyruvate	2	4.27 ± 0.07 (8)	146
Lactate	2	3.70 ± 0.15 (9)	130
Succinate	5	2.37 ± 0.21 (2)	83
Glutamate + malaat	5	2.41 ± 0.13 (2)	84
α-Ketoglutarate	5	2.69 ± 0.26 (2)	94

[a] Mean values ± SE (number of experiments, each performed in triplicate).

[b] Number of experiments, each performed in triplicate.

a sample of the mitochondrial suspensions was mixed with an equal volume of ice-cold 20% trichloroacetic acid. After centrifugation, glucose, α-ketoglutarate and pyruvate concentrations were measured by standard enzymatic procedures (Bergmeyer, 1974), in the supernatants neutralized with 1.75 M potassium phosphate. The mean consumption of the respective compounds in triplicate incubations was calculated from thè concentrations before and after the experiment. All results are expressed in micromoles per milligram of mitochondrial protein per 30 min.

Statistical analysis. Student's *t* test (De Jonge, 1960) were used to calculate one-side significances of differences between values of treated and control cell suspensions.

RESULTS

Respiration of Rat Thymocytes in the Presence of Various Substrates

To study the effects of organotin compounds on the energy metabolism, the capability of these cells to convert various substrates was determined by measuring the stimulation of the endogenous respiration. From Table 1 it can be seen that the oxygen consumption of these cells is stimulated by various carbohydrates. However, it was not possible to stimulate endogenous respiration with mitochondrial substrates such as succinate, α-ketoglutarate, or glutamate plus malate. Glucose and pyruvate stimulated endogenous respiration with about 45%, lactate with 30%.

Effects of Various Dialkyltin Compounds on the Aerobic and Anaerobic Glucose Metabolism of Rat Thymus Cells

Aerobic incubations. In rat thymocytes incubated with glucose as substrate, the various organotin compounds induced a dose-related increase of the consumption of glucose and the production of lactate and pyruvate. Glucose uptake was already increased at DETC, DBTC, DOTC, and DMTC concentrations of 0.5, 0.5, 5, and 10 μM, respectively. A maximal stimulation of the glucose consumption was observed at concentrations of 5 and 10 μM DBTC and DETC, respectively, and at much higher concentrations of 120 μM DOTC and DMTC. At higher exposure levels glucose consumption progressively decreased. The uptake was almost completely inhibited at a concentration of 60 μM DETC and DBTC. DMTC also reduced the glucose uptake but at a relatively high concentration of 240 μM. In case of DOTC the glucose consumption was still stimulated at a concentration of 240 μM, the highest level that could be achieved in our cell culture medium.

The increased amount of glucose consumed by thymocytes upon incubation with the organotin compounds, was not completely metabolized but largely converted to lactate and pyruvate (Fig. 1). So it is converted by the glycolytic pathway whereas it is hardly oxidized by the TCA cycle. The lactate and pyruvate production was stimulated maximally at organotin concentrations that induced a maximum glucose consumption.

Oxygen consumption of rat thymocytes was dose relatedly decreased. It was markedly reduced at concentrations above those inducing a maximum stimulation of the substrate uptake. At maximum stimulation the oxygen consumption was never reduced by more than 30% and at lower levels it was slightly diminished only. This is remarkable, since the accumulation of lactate and pyruvate showed that the increased amount
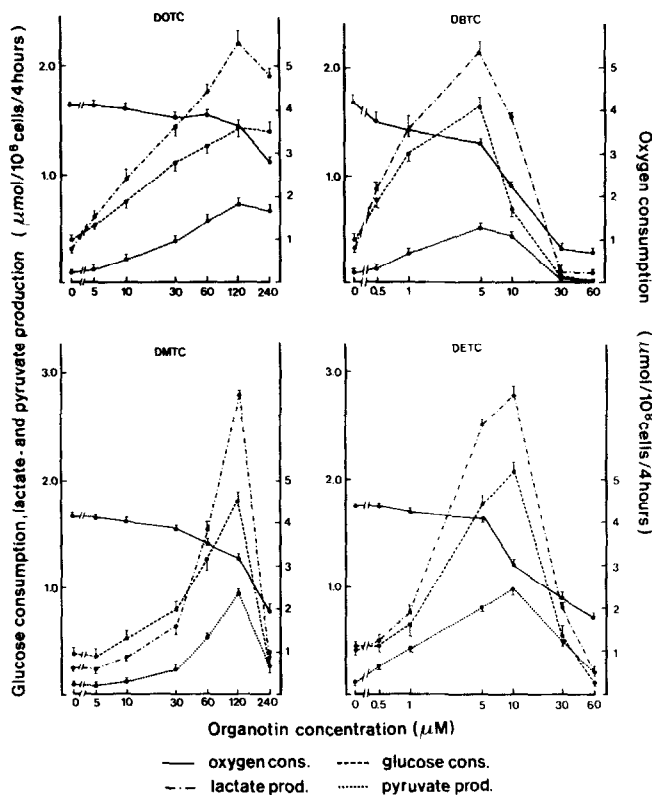
FIG. 1. Consumption of glucose and oxygen and the production of lactate and pyruvate of isolated rat thymocytes incubated with various concentrations of DOTC, DBTC, DETC, and DMTC for 4 hr. Results are means ± SE of at least three incubations each performed in triplicate.

of glucose consumed is hardly metabolized oxidatively.

The number and viability of rat thymocytes was not affected during the 4-hr incubation period. At levels up to those inducing a maximum stimulation of the substrate consumption, the number and viability of the thymocytes was always comparable to the controls. Above a maximum stimulation cell survival declined but was never reduced by more than 10%. Therefore, the progressive fall in glucose and oxygen consumption is not caused by a decrease of the cell survival.

Anaerobic incubations. In comparison with the aerobic condition the glucose consumption of thymocytes is fourfold increased by anaerobiosis (Table 2). Therefore thymus cells possess a dominating oxidative metabolism.

The organotin compounds did not affect the anaerobic glucose metabolism up to concentrations which induced a maximum stimulation of glucose metabolism in the aerobic systems (120 μM DOTC, 5 μM DBTC, 10 μM DETC, and 120 μM DMTC). A further increase of the concentration of DBTC and DETC dose relatedly diminished the glucose consumption of thymus cells as was found in the aerobic system. DOTC and DMTC were not tested at higher concentrations.

Independently from the dose of the organotins the glucose was always largely converted to lactate, so there is no effect of these organotin compounds on the glycolytic pathway of thymus cells.

Cell viability, as scored by the nigrosine exclusion test, was never reduced by more than 10% after the 4-hr incubation period.

TABLE 2

Effect of Various Concentrations of DOTC, DBTC, DETC, and DMTC on Glucose Consumption and Lactate Production of Isolated Rat Thymus Cells Utilizing 2 mM Glucose as Substrate under Anaerobic Conditions[a]

Compound	Concentration (μM)	μmol/10⁶ cells/4 hr	
		Glucose consumption	Lactate production
Control (aerobiosis)		0.42 ± 0.02 (15)	0.33 ± 0.03
Control (anaerobiosis)		1.60 ± 0.07 (9)	2.66 ± 0.08
DOTC	120	1.58 ± 0.10 (9)	2.88 ± 0.07
DBTC	60	0.29 ± 0.10[b] (3)	0.39 ± 0.04[b]
DBTC	30	0.75 ± 0.02[b] (3)	1.76 ± 0.10[b]
DBTC	10	1.10 ± 0.07[b] (3)	2.30 ± 0.11[b]
DBTC	5	1.64 ± 0.09 (3)	2.77 ± 0.12
DETC	60	0.61 ± 0.08[b] (3)	0.94 ± 0.03[b]
DETC	30	0.99 ± 0.09[b] (2)	2.10 ± 0.05[b]
DETC	10	1.69 ± 0.03 (2)	3.00 ± 0.09
DETC	5	1.67 ± 0.11 (2)	2.92 ± 0.05
DMTC	120	1.54 ± 0.07 (5)	2.82 ± 0.07

[a] Mean values ± SE (number of experiments, each performed in triplicate).
[b] $p < 0.001$.

Effects of DOTC and DBTC on Pyruvate- and Lactate Metabolism of Rat Thymus Cells

Pyruvate metabolism. In the presence of DOTC and DBTC the pyruvate consumption of rat thymus cells was dose relatedly decreased (Table 3). It was already reduced at levels of 10 μM DOTC and 1 μM DBTC and progressively diminished to 35 and 29% of the control values at concentrations of 120 μM DOTC and 10 μM DBTC, respectively. Also the lactate production was reduced absolutely by the organotin compounds, but it was relatively increased as can be seen from the dose-related increase of the lactate–pyruvate ratio.

Oxygen consumption was decreased in a dose-related fashion. At concentrations 5 μM DBTC and 120 μM DOTC, which both induce a maximal stimulation of the glucose consumption, the oxygen uptake was re-

TABLE 3

Effect of Various Concentrations of DOTC and DBTC on Oxygen- and Pyruvate Consumption and Production of Lactate by Isolated Rat Thymus Cells Utilizing 2 mM Pyruvate as Substrate under Aerobic Conditions[a]

Concentration (μM)	Oxygen consumption	Pyruvate consumption	Lactate production	Lactate/pyruvate ratio
DOTC				
Control	4.24 ± 0.06 (5)	1.99 ± 0.13	0.46 ± 0.03	0.23
120	2.25 ± 0.08[b] (5)	0.57 ± 0.14[b]	0.22 ± 0.03[b]	0.39
30	3.07 ± 0.12[b] (5)	1.15 ± 0.14[b]	0.33 ± 0.04[b]	0.29
10	3.54 ± 0.15[b] (5)	1.41 ± 0.15[d]	0.37 ± 0.03[c]	0.26
5	3.94 ± 0.10[d] (4)	1.82 ± 0.19	0.34 ± 0.07	0.19
DBTC				
Control	4.30 ± 0.15 (5)	1.99 ± 0.11	0.49 ± 0.02	0.25
10	1.27 ± 0.10[b] (5)	0.70 ± 0.09[b]	0.23 ± 0.02[b]	0.33
5	2.37 ± 0.14[b] (4)	0.81 ± 0.10[b]	0.24 ± 0.03[b]	0.30
1	3.89 ± 0.11[b] (5)	1.69 ± 0.12[c]	0.43 ± 0.04[d]	0.25
0.5	4.23 ± 0.09 (5)	1.83 ± 0.09	0.47 ± 0.03	0.26

Note. Results are expressed in μmol/10⁸ cells/4 hr.
[a] Mean values ± SE (number of experiments, each performed in triplicate).
[b] $p < 0.001$.
[c] $p < 0.01$.
[d] $p < 0.05$.

TABLE 4

THE EFFECTS OF VARIOUS CONCENTRATIONS OF DOTC AND DBTC ON OXYGEN- AND LACTATE CONSUMPTION
AND PRODUCTION OF PYRUVATE BY ISOLATED RAT THYMOCYTES UTILIZING 2 mM LACTATE AS SUBSTRATE
UNDER AEROBIC CONDITIONS[a]

Concentration (μM)	Oxygen consumption	Lactate consumption	Pyruvate production	Pyruvate/ lactate ratio
		DOTC		
Control	3.72 ± 0.04 (4)	1.30 ± 0.10	0.36 ± 0.02	0.28
120	2.90 ± 0.04[b] (4)	1.25 ± 0.09	0.74 ± 0.04[b]	0.59
30	3.31 ± 0.09[c] (4)	1.31 ± 0.12	0.64 ± 0.04[b]	0.49
10	3.43 ± 0.06[c] (4)	1.23 ± 0.07	0.52 ± 0.02[b]	0.42
5	3.81 ± 0.04 (4)	1.21 ± 0.08	0.28 ± 0.02	0.23
		DBTC		
Control	3.63 ± 0.07 (4)	1.01 ± 0.10	0.33 ± 0.01	0.33
10	1.47 ± 0.07[b] (4)	0.55 ± 0.08[b]	0.53 ± 0.02[b]	0.96
5	2.80 ± 0.10[c] (3)	0.86 ± 0.10	0.56 ± 0.02[b]	0.65
1	3.95 ± 0.11[c] (4)	0.97 ± 0.08	0.36 ± 0.01[c]	0.37
0.5	3.51 ± 0.13 (4)	1.09 ± 0.19	0.35 ± 0.01	0.32

Note. Results expressed in μmol/10[6] cells/4 hr.
[a] Mean values ± SE (number of experiments, each performed in triplicate).
[b] $p < 0.001$.
[c] $p < 0.01$.

duced to 55 and 53%, respectively. At a higher concentration of 10 μM DBTC the inhibition of the oxygen consumption was more pronounced, as was also found with glucose as substrate.

Cell viability was decreased at a concentration of 10 μM DBTC only, but never exceeded a 10% reduction.

Lactate metabolism. The consumption of lactate by rat thymus cells was only diminished upon incubation with 10 μM DBTC (Table 4). All other concentrations of DBTC and DOTC tested did not reduce the lactate consumption, which is in contrast with the effects of these compounds on the pyruvate metabolism of thymus cells (Table 3). Although the consumption of lactate was not affected, the metabolism was markedly changed, as can be seen from the dose-related increase of the pyruvate production (Table 4). At levels of 1 μM DBTC and 10 μM DOTC the pyruvate production exceeded the controls, and it progressively increased to 211 and 170% of the control

values at concentrations of 120 μM DOTC and 5 μM DBTC, respectively. The pyruvate–lactate ratio's indicate that the amount of lactate converted to pyruvate increased in a dose-related fashion.

Oxygen consumption was dose relatedly decreased. At concentrations of 5 μM DBTC and 120 μM DOTC, the oxygen consumption was reduced to 77 and 78%, respectively. These effects were comparable to those found with glucose, but were less pronounced than those seen with pyruvate. A sharp fall in oxygen consumption was seen at a concentration of 10 μM DBTC, as was also found with the other substrates used. Only at this concentration cell viability was slightly reduced.

Effects of DBTC on Pyruvate- and α-Keto glutarate Metabolism of Rat Liver Mitochondria

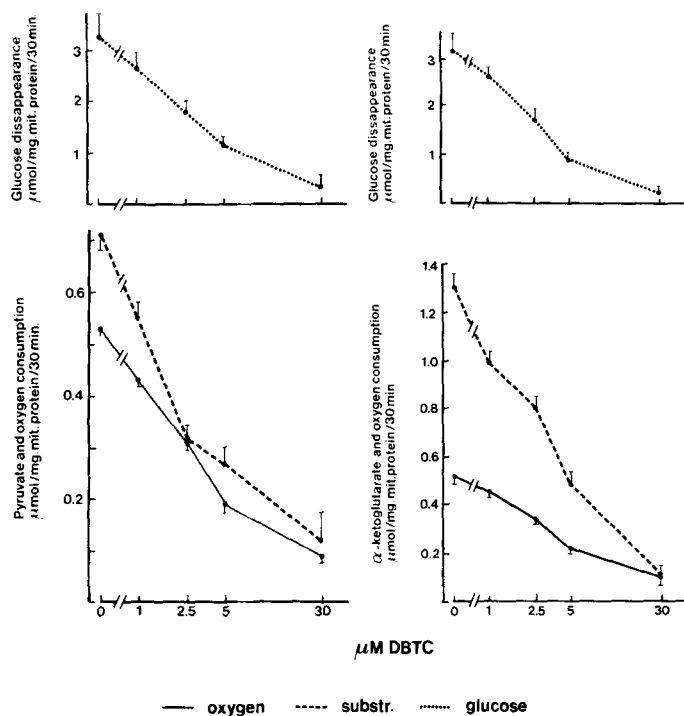In the presence of DBTC the consumption of pyruvate and α-ketoglutarate by rat

FIG. 2. Consumption of pyruvate, α-ketoglutarate, and oxygen of rat liver mitochondria incubated with various concentrations of DBTC for 30 min, and the disappearance of glucose from the medium during the incubation. Results are means ± SE of two incubations each performed in triplicate.

liver mitochondria was dose relatedly reduced (Fig. 2). It was already decreased at levels of 1 μM DBTC and progressively diminished to 17 and 9% of the control values, respectively, at a concentration of 30 μM DBTC.

During the incubation with the mitochondrial substrates the glucose consumption decreased in a dose-related manner comparable to the inhibition of the substrate consumption. Since in the presence of ATP, glucose will be converted to glucose 6-phosphate by hexokinase, the decline of the glucose concentration is a measure of the ATP producing capacity of the mitochondria. So, the ATP production of rat liver mitochondria is also dose relatedly decreased by DBTC.

The oxygen consumption of rat liver mitochondria was also diminished by DBTC in a dose-related fashion. At a concentration

of 30 μM DBTC the oxygen consumption was only 17% of the control values.

DISCUSSION

From the present study it appears that di-alkyltin compounds, DOTC, DBTC, DETC, and DMTC, induce a similar impairment of the energy metabolism of rat thymocytes, but at different concentrations. DBTC and DETC were the most toxic compounds, inducing a maximum stimulation of glucose consumption at levels of 5 and 10 μM, respectively. At higher exposure levels a marked inhibition of thymocyte metabolism was observed. Together with a sharp fall in stimulation of the glucose consumption, the oxygen consumption of thymocytes was markedly inhibited and also a decrease of cell viability was noted.

Under anaerobic conditions the glucose

metabolism of rat thymocytes was not affected up to levels inducing a maximum glucose consumption in the aerobic system. Therefore it is concluded, that the glycolytic pathway is not affected by the dialkyltin compounds. Since pyruvate and lactate accumulated in thymocytes incubated with glucose, the oxidation of the end products of the glycolytic pathway in the TCA cycle is probably inhibited by dialkyltin compounds. Aldridge and Cremer (1955) already noted pyruvate accumulation in rat brei oxidizing lactate as substrate in the presence of DETC. They suggested a binding of the dialkyltin compounds with the dithiol group of lipoic acid which is a protein bound cofactor of the pyruvate dehydrogenase system and also of the α-ketoglutarate dehydrogenase complex (Leninger, 1972). It is also possible that lipoyl dehydrogenase, an enzyme of these α-keto acid dehydrogenase complexes, containing two vicinal thiol groups (Massey and Veeger, 1961), is inhibited by dialkyltin compounds as was found for arsenite (Massey and Veeger, 1960). *In vitro* dialkyltins readily bind dithiol groups (Aldridge and Cremer, 1955; Cain et al., 1977) and the toxicity of dialkyltins in rats was markedly reduced by treatment with 2,3-mercaptopropanol. So it seems reasonable that the pyruvate dehydrogenase complex is inhibited by dialkyltin compounds.

Additional support for the inhibition of the pyruvate dehydrogenase complex was obtained from incubations of rat thymocytes with lactate and pyruvate. In the presence of DOTC and DBTC the pyruvate consumption was dose relatedly decreased until 29 and 33% of the controls at concentrations of 120 μM DOTC and 10 μM DBTC, respectively. The lactate consumption was only decreased at a level of 10 μM DBTC but it was converted to pyruvate in a dose-dependent manner as can be seen from the progressive increase of the pyruvate–lactate ratio. Probably the uptake of lactate

is not reduced since the conversion of lactate to pyruvate is energetically favourable when the entrance of pyruvate into the TCA cycle is blocked. Also in rat liver mitochondria a dose-related decrease of pyruvate oxidation was observed in the presence of DBTC. A comparable inhibition of α-ketoglutarate oxidation was found, indicating that also α-ketoglutarate dehydrogenase activity is inhibited by dialkyltin compounds. This if further supported by the accumulation of α-ketoglutarate observed in mitochondria incubated with L-glutamate (Seinen and Penninks, 1979). In rat thymocytes inhibition of the α-ketoglutarate dehydrogenase system could not be shown, since the endogenous metabolism of thymocytes was not stimulated by intermediates of the TCA cycle, which probably do not penetrate the cell membrane.

In comparison with the effects on substrate metabolism the effect of dialkyltin compounds on the oxygen consumption of rat thymocytes was less pronounced. It was markedly reduced at relatively high exposure levels only. In thymocytes incubated with glucose or lactate the oxygen consumption was only decreased by 24 and 22% in the presence of 5 μM DBTC or 120 μM DOTC, respectively, which inhibited the TCA cycle activity almost completely. With pyruvate as substrate the oxygen consumption was reduced by 45 and 47%, respectively. The different inhibition of oxygen consumption observed in thymocytes incubated with glucose or lactate versus pyruvate may be explained by the different production of NADH which is oxydized in the respiratory chain. Otherwise it remains difficult to understand why the oxygen consumption is still about 55% of the controls. Possibly endogeneous substrates, such as fatty acids may deliver intermediates for the electron transport chain. Also the unique phenomenon of the thymocyte nuclei to produce ATP by an electron transport mechanism may attribute to the

oxygen consumption of the thymocytes (Osawa *et al.*, 1956; McEwen *et al.*, 1963; Betel *et al.*, 1967).

After a 4-hr incubation period the viability of thymocytes was not affected by concentrations of the dialkyltin compounds up to levels inducing a maximum stimulation of the glucose consumption. At higher exposure levels cell survival declined but was still high in spite of a serious disturbance of the thymocyte metabolism. After a longer incubation period of 24 hr thymocyte survival markedly decreased, even at a lower concentration of 1.5 μM DBTC (Seinen *et al.*, 1977). Therefore, the absence of organotin induced cell death as measured with the dye exclusion test will not exclude a cytotoxic action of these compounds.

In vivo, dialkyltin compounds induce thymus involution in rats (Seinen and Willems, 1976; Seinen *et al.*, 1977; Seinen, 1980; Seinen and Penninks, 1979). In the thymus bone-marrow-derived stem cells migrate into the thymic cortex where, under the stimulatory and perhaps regulatory influence of thymic epithelial cells, cell division and differentiation begin, giving rise to the forming of various subclasses of thymus-derived T-lymphocytes (Stutman, 1978). The mitotic activity within the thymus is very high and the cell cycle time of thymic lymphocytes is short, in the range of 8.5–9.5 hr for mouse thymocytes (Bryant, 1971). As a consequence of a limited energy supply, the mitotic activity is diminished, which can account for a reduction in thymus weight. So, the *in vivo* observed thymus involution may be related to the *in vitro* effects of dialkyltin compounds on glucose metabolism of thymocytes. However, *in vivo* only dialkyltin compounds with chain length of 4–8C atoms induced a remarkable lymphocytotoxicity in thymus and thymus-dependent areas of peripheral lymphoid organs without compromising other organ systems. In contrast, both in the glucose metabolism studies and in the

lymphocyte transformation tests (Seinen and Penninks, 1979) only slight differences in *in vitro* effects were observed between the various dialkyltin compounds with chain length of 1–8C atoms. Between the homologs there exists marked differences in water and fat solubility. The lower homologs (1–3C atoms) are more water soluble whereas the higher homologs (4–8C) are more fat soluble. The fat–water partition of drugs is an important factor which determines membrane passage and the distribution of compounds throughout the body. Therefore, the absence of thymus effects of the lower homologs may be related to differences in distribution pattern of the various dialkyltin compounds.

When the basis of cytotoxicity will be an impairment of cell energetics as is demonstrated in this study, the question remains why especially thymocytes are affected *in vivo*. The most simple explanation of thymocyte selectivity could be an accumulation of the organotins in the thymus as was shown by Seidler *et al.* (1971) after a single oral intubation of [^{14}C]DOTC in rats. To elucidate the significance of differences in distribution for the selective lymphocytotoxicity *in vivo*, pharmacodynamic studies with various labeled dialkyltin compounds are in progress.

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