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EFFECT OF 1,1,1-TRICHLOROETHANE ON MITOCHONDRIAL METABOLISM

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Abstract—The effect of 1,1,1-trichloroethane (TCE) on rat liver (and heart) mitochondrial metabolism was assessed by studying the respiratory control characteristics associated with ATP synthesis from added ADP as well as low level Ca^{2+} uptake. With pyridine nucleotide-linked substrates, there is a marked decline in State 3 (ADP) respiration (I_{50} = 0·65 μmoles TCE/mg of mitochondrial protein). This respiratory inhibition was found to be due to interruption of electron transfer at the rotenone-sensitive site on the electron transport chain. Interestingly, the ADP/O ratio is largely unaffected. In contrast, succinate-linked State 3 (ADP) respiration is not inhibited by TCE (in this same concentration range), but there appears to be an apparent uncoupling due to enhanced State 4 (ADP) respiration. This latter effect is due to the release of an ATPase activity dependent on exogenous Mg^{2+}. In the absence of exogenous Mg^{2+}, ATP synthesis and ATPase activity are largely unaffected by TCE. From studies on low level Ca^{2+} uptake, the data indicate that the halocarbon alters the passive permeability characteristics of the mitochondrion to Ca^{2+} and H^+, but the Ca^{2+} binding and sequestration mechanisms are unaffected. In conclusion, the data provide a mechanistic basis for the previously described, TCE-induced depression of myocardial respiration and the alteration in myocardial contractility observed during acute exposure to this drug.

1,1,1-Trichloroethane (TCE)* is a halogenated hydrocarbon of increasing commercial and toxicological importance. It is rapidly replacing commercial use of carbon tetrachloride and other organic solvents because of its reported low toxicity [1, 2], and it has been utilized in numerous, home-use products, including inhalant decongestant sprays [3].

Recent reports of its toxic effects on man [4–6] and under experimental conditions [7, 8] suggest that the toxic effects of this compound deserve further scrutiny. We have reported [8] that, in the anesthetized dog, acute inhalation of TCE depresses the cardiovascular system. The depression occurs in a biphasic manner with the initial event being reflex peripheral vasodilation, followed by a suppression of myocardial contractility.

In view of the report by Krantz *et al.* [7] that TCE inhibits the respiration of myocardial tissue slices, we propose to determine whether TCE-induced alterations in mitochondrial function could provide a basis to explain the observed depression of myocardial contractile function.

METHODS

Female albino rats (250–350 g) were fasted overnight. Liver and heart mitochondria were prepared according to standard techniques [9, 10] and were used immediately following isolation. Because of the relatively low quantity of heart mitochondria obtained from individual rats, it was not possible to complete dose–response studies with these particles, nor were suitable replicate trials obtained; therefore, these data are not included here. However, in each experiment to be described, heart mitochondria responded in a fashion (and sensitivity) essentially identical to that observed with liver mitochondria.

Frozen–thawed mitochondria were prepared by at least three successive freeze–thaw cycles (20° ↔ + 23°). These mitochondria respired essentially as uncoupled mitochondria, and their insensitivity to added DNP and ADP was assessed in each preparation.

Respiratory control characteristics were studied polarographically at 30°, as described previously [9, 10]. ADP-dependent respiration was measured in a standard medium (vol. 1·6 ml) containing: mannitol (0·150 M), sucrose (0·060 M), $MgCl_2$ (0·0033 M), HEPES (*N*-2-hydroxy ethylpiperazine *N'*-2-ethanesulfonate) buffer (pH 7·4, 0·01 M), potassium phosphate (0·010 M), EDTA (ethylenediaminetetraacetic acid, 0·001 M), substrates (0·01 M) and other cofactors as noted in the results.

Low level Ca^{2+} uptake, Ca^{2+} binding and concomitant H^+ efflux were measured in the polarograph cuvette according to the protocols of Rossi and Lehninger [11], except that HEPES buffer (pH 7·4) was used. Respiratory rates are measured as nanogram atoms (n-atoms) oxygen/mg of mitochondrial protein/min.

In both ADP- and Ca^{2+}-dependent respiratory control cycles, the burst of respiration associated with addition of ADP (or Ca^{2+}) is designated State 3 (ADP) or State 3 (Ca) [9]. In like manner, the subsequent controlled phase of mitochondrial respiration is designated as State 4 (ADP) or State 4 (Ca).

Mitochondrial ATPase activity was measured according to the technique of Lardy and Wellman [12] in both intact and frozen–thawed mitochondria at 30° in a final volume of 1·0 ml.

The initial rate of mitochondrial swelling associated with ATP-driven Ca^{2+} (acetate) uptake was measured

* Abbreviations: ADP (adenosine diphosphate), ATP (adenosine triphosphate), NAD (nicotinamide adenine dinucleotide). Other abbreviations are as noted in the text.

at 30° according to the method of Packer [13] at 520 nm. The reaction medium was identical to that used in the respiratory studies except that phosphate, EDTA and $MgCl_2$ were absent, and 0·5 mM calcium acetate was present in a final volume of 1·0 ml. Swelling was initiated by addition of 2·0 mM ATP and the initial rate of swelling is reported as Δ O. D./mg of mitochondrial protein/min.

Mitochondrial compartmentation experiments were carried out in the polarograph cuvette (*vide supra*) according to the procedure described by Harris and Van Dam [14]. After incubation (as noted in the Results), the mitochondria were separated from the reaction medium by centrifugation through a silicone layer (GE F-50, General Electric Co., Scenectady, N.Y.) using a Beckman Microfuge.

Radioactivity measurements were carried out in Aquasol (New England Nuclear, Boston, Mass.) and counted in a Nuclear Chicago Mark II liquid scintillation counter. Mitochondrial protein was measured by the biuret method [15].

All reagents were of the highest purity available. 2-4-Dinitrophenol (Sigma) was recrystallized from ethanol. The ADP (Sigma) concentration was estimated using the extinction coefficient $E_{mM}^{259nm} = 15{\cdot}4\ mM^{-1}$. 1,1,1-Trichloroethane was obtained from various commercial preparations as described previously [8]. Briefly, since dioxane is the major contaminant of commercial preparations of TCE, (2–3 per cent; P. Herd, unpublished observation), the mixture was washed sequentially with several (at least ten v/v) changes of water followed by concentrated sulfuric acid, and then again with water until all acid had been removed. The resultant material was at least 99·5 vol% TCE as assessed by vapor phase chromatography. TCE was diluted in 95% ethanol (final concentration <1%).

RESULTS

The interaction between TCE and the ADP-dependent respiratory response associated with the oxidation of pyridine nucleotide-linked substrates (Fig. 1) is marked by a decline in State 3 (ADP) respiration. Similarly, 2,4-dinitrophenol-stimulated respiration also declined (not shown). At low concentrations of TCE, State 4 (ADP) respiration was enhanced; however, this stimulation was followed by respiratory inhibition at concentrations greater than 0·80 μmole TCE/mg of mitochondrial protein. Addition of oligomycin to the mitochondria respiring in State 4 (ADP) resulted in a respiratory rate which is independent of the TCE concentration in the range shown. Pyridine nucleotide-linked respiration ceased above 3·5 μmoles TCE/mg of mitochondrial protein.

In Table 1, it is seen that the stoichiometry of oxidative phosphorylation is only slightly affected by TCE up to 2·0 μmoles TCE/mg of mitochondrial protein (above which, precise measurement was not possible).

The concentration of TCE producing 50 per cent inhibition of State 3 (ADP) respiration is 0·65 μmole TCE/mg of mitochondrial protein. Identical results were obtained with frozen–thawed mitochondria supplemented with NAD and cytochrome c (not shown), suggesting that neither cofactor nor substrate availability was a factor affecting the TCE-induced inhibition of State 3 (ADP) respiration.

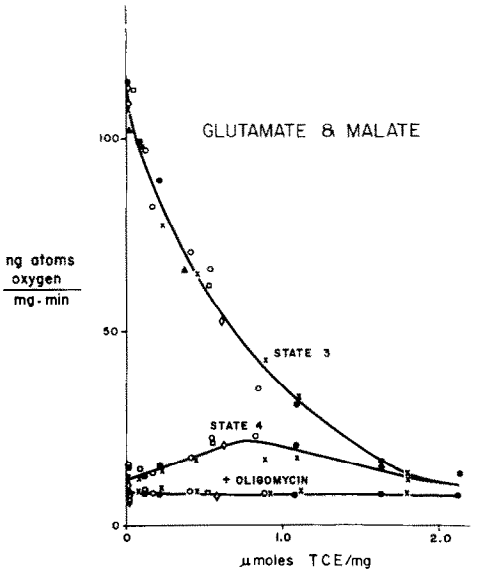

Fig. 1. Effect of TCE on ADP-dependent respiratory control characteristics with glutamate plus malate as substrate. See Methods for experimental details. Each datum point reflects the average of at least two trials at the dose of TCE. The various symbols reflect different preparations of mitochondria. The lowest trace (designated as + oligomycin) reflects the respiratory rate obtained after addition of 0·5 μg oligomycin to the mitochondria (2·4–3·0 mg) respiring at the State 4 (ADP) rate. The curve is drawn as a best fit by eye.

A variety of preincubation conditions were attempted to protect the mitochondria against this effect of TCE. Among the reagents tested which were found to be ineffective were: defatted serum albumin (up to 2 mg/ml), NAD (1·0 mM), cytochrome c (250 μg/ml), coenzyme Q_{10} (25 μg/ml), ATP (2·0 mM), Mg^{2+} (6·0 mM), EDTA (1·5 mM) and K^+ (20 mM).

Vitamin K_3, however, which is reported to shunt electron flow around the rotenone-sensitive site on the electron transport chain [16], effectively prevented the TCE-induced inhibition of pyridine nucleotide-linked respiration, as seen in Fig. 2 (compare traces b and c). These results are consistent with the lipophilic character of the rotenone-sensitive site on the electron transport chain [17] and its reported sensitivity

Table 1. Effect of 1,1,1-trichloroethane (TCE) on the ADP/O ratio in rat liver mitochondria*

Concentration of TCE (μmoles/mg mitochondrial protein)	N†	Glutamate + malate	N	Succinate
0·0–0·5	10	2·74 ± 0·35‡	6	1·90 ± 0·10
0·5–1·0	10	2·74 ± 0·35	6	1·90 ± 0·10
1·0–1·5	6	2·55 ± 0·45	6	1·90 ± 0·10
1·5–2·3	Not detectable		6	1·75 ± 0·25

* ADP/O values were obtained from the polarographic trace of oxygen uptake as described by Estabrook [10].

† N = number of mitochondrial preparations tested.

‡ ADP/O values ± S.D. of appropriate replicate samples. With pyridine nucleotide-linked substrates (glutamate + malate), the State 3 (ADP) to State 4 (ADP) transition is not sufficiently distinct above 1·5 μmoles TCE/mg of mitochondrial protein to provide a suitable tracing for analysis of ADP/O.

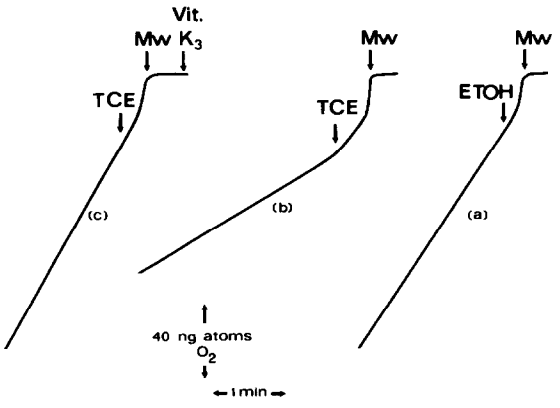

Fig. 2. Effect of vitamin K_3 on the TCE-induced inhibition of NAD-linked respiration. Frozen–thawed mitochondria were incubated in a standard reaction medium (see Methods) to which was added cytochrome c (250 μg) and NAD (0·5 mM). Also, the following reagents were added to 2·4 mg mitochondria (Mw): ethanol as a control (trace a), TCE (traces b and c, 1 μmole/mg of mitochondrial protein) and vitamin K_3 (trace c, 5 μM).

to a variety of organic reagents, including other halogenated hydrocarbons [17, 18].

The respiratory control characteristics associated with succinate-linked respiration revealed a much different response to TCE. In Fig. 3, concentrations of TCE equivalent to those used with pyridine nucleotide-linked substrates did not evoke an alteration in State 3 (ADP) respiration (or DNP-stimulated respiration; not shown), ADP/O (Table 1) or in the oligomycin-insensitive respiratory rate. However, the State 4 (ADP) respiratory rate is markedly enhanced in a concentration-dependent manner. After the addition of oligomycin (or atractyloside), the State 4 (ADP) respiratory rate is depressed to a TCE-independent respiratory rate, which suggests that this enhanced State 4 (ADP) respiration is due to an augmented mitochondrial ATPase activity [9].

* Adenylate kinase (EC 2.7.4.3), AMP + ATP = 2 ADP; ATP synthetase, 2 ADP + Pi = 2 ATP.

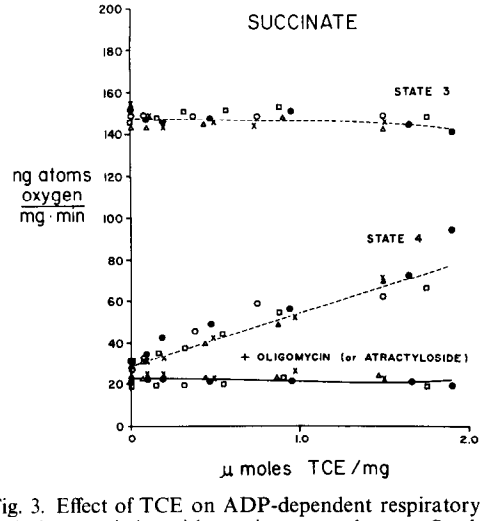

Fig. 3. Effect of TCE on ADP-dependent respiratory control characteristics with succinate as substrate. See legend to Fig. 1 for additional experimental details.

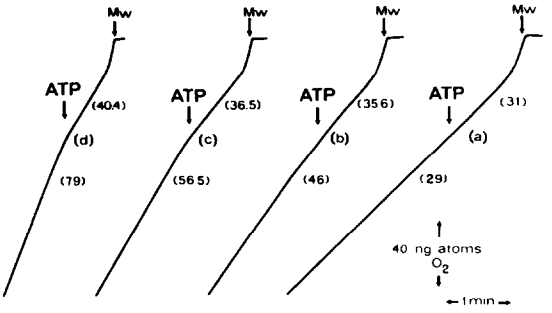

Fig. 4. Effect of TCE on ATP-dependent, succinate-linked respiration. See Methods for experimental details. The reaction contained: 0·2 μmole TCE/mg of mitochondrial protein in trace b, 0·5 μmole TCE/mg of mitochondrial protein in trace c, and 1·0 μmole TCE/mg of mitochondrial protein in trace d. In trace a, 95% ethanol was added as the control. In all traces the ATP concentration was 0·5 mM.

At higher concentrations of the drug (2·6 μmoles TCE/mg of mitochondrial protein), respiratory control is lost, and there follows a rapid respiratory inhibition which is complete at 4·5 μmoles TCE/mg of mitochondrial protein.

The dose-dependent increase in State 4 (ADP) respiration was mimicked by addition of ATP to the mitochondria respiring in the presence of varying levels of TCE (Fig. 4). This stimulatory effect of ATP requires the presence of exogenous Mg^{2+} (Fig. 5a vs 5b). In trace c of Fig. 5, it is seen that in the absence of exogenous Mg^{2+}, the capacity of the mitochondria to synthesize ATP from AMP (or ADP) is not impaired by the presence of TCE, and this oxidative phosphorylation is supported by endogenous Mg^{2+}, since the respiratory cycle initiated by adding AMP (or ADP) is inhibited by the divalent cation chelator, EDTA, and this inhibition is reversed by exogenous Mg^{2+} (Fig. 5d). It is known that phosphorylation of AMP to ATP requires the activity of two Mg^{2+}-dependent enzymes,* the adeylate kinase of the intermembrane space [19] and the ATP synthetase. In view of these considerations, the data are interpreted to suggest that the endogenous Mg^{2+} is either inadequate or inaccessible to support this TCE-induced ATPase activity which requires exogenous Mg^{2+}.

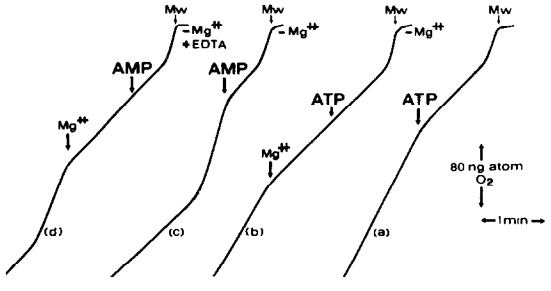

Fig. 5. Role of Mg^{2+} in the TCE-induced, ATP-dependent stimulation of mitochondrial respiration. See Methods for standard reaction medium to which was added: $MgCl_2$ (3·3 mM, traces b and d), ATP (0·5 mM, traces a and b), AMP (0·5 mM, traces c and d), EDTA (1·0 mM, trace d); 1 μmole TCE/mg of mitochondrial protein was added in each experiment to 2·5 mg of mitochondria.

Table 2. Effect of 1,1,1-trichloroethane (TCE) on mitochondrial, oligomycin-insensitive ATPase activity

Preparation*	N	Activity†
Basal	4	0·123 ± 0·011
+ Oligomycin (0·2 μg)	4	0·016 ± 0·013
+ 2,4-Dinitrophenol (0·5 mM)	2	0·016
+ TCE (1 μmole/mg mitochondrial protein)	4	0·084 ± 0·010
+ Oligomycin (0·2 μg)	3	0·084 (0·82 0·85)

* Two mg frozen–thawed mitochondria/1·6 ml was preincubated with 6 mM Mg-ATP (see Methods).

† Expressed as μmoles ATP hydrolyzed/mg × min.

Results obtained in studies on the effect of TCE on oligomycin-insensitive ATPase activity (Table 2) suggest that TCE releases a latent ATPase activity. Frozen–thawed (hence, uncoupled) mitochondrial ATPase activity is inhibited by oligomycin; however, when TCE (1 μmole/mg of mitochondrial protein) is added, there is a 5-fold increase in the oligomycin-insensitive ATPase activity, and further additions of oligomycin are without effect. This ATPase activity is either related to a release of oligomycin sensitivity or is due to the release of a latent, unrelated ATPase activity. At present, the data favor the latter possibility, in view of the requirement for exogenous Mg^{2+} to observe this effect.

A functional correlate of the enhanced mitochondrial ATPase activity is seen in Table 3 where the interaction of Mg^{2+} and TCE on the distribution of mitochondrial water is reported. During State 4 (ADP) respiration in the presence of TCE and Mg^{2+}, the sucrose permeant space, an indicator of the volume of the intermembrane space of the mitochondrion [20], collapses and there is a concomitant increase in the sucrose impermeant space which corresponds to the mitochondrial matrix compartment [20]. In contrast, in the absence of added Mg^{2+}, there is a relatively small shift in mitochondrial water between these two compartments and the volume change is opposite to that seen in the presence of exogenous Mg^{2+}.

Mitochondrial Ca^{2+} uptake. In Fig. 6, results are presented from an experiment (N = 6) in which low level Ca^{2+} uptake was assessed in the presence of varying concentrations of TCE. The response of State 3 (Ca) is biphasic; initially there is a decline in the respiratory rate at low doses of the drug, which is followed by marked stimulation. This latter response is the result of a dose-dependent increase in State 4 (Ca) respiration and, at 1·5–2·0 μmoles TCE/mg of mitochondrial protein, the State 3 (Ca) and State 4 (Ca) rates become continuous,* suggesting an uncoupling of respiration from the energy-linked uptake of Ca^{2+}. However, addition of a specific inhibitor of Ca^{2+} uptake, La^{3+} [21], during State 4 (Ca) respiration returned the respiratory rate to that recorded before the addition of Ca^{2+}, i.e. the pre-State 3 (Ca) respiratory rate. It is of interest to note that essentially identical results are obtained when Ca^{2+}-induced respiratory cycles are studied in the presence of exogenous Mg^{2+} (P. Herd, unpublished observations). These findings suggest that the enhanced State 4 (Ca) respiration is due to the re-uptake of Ca^{2+} which had been sequestered during State 3 (Ca), and imply that the passive efflux of this sequestered Ca^{2+} is enhanced by TCE.

* The concentration of TCE at which State 3 (Ca) and State 4 (Ca) respiratory rates become continuous depends, in part, on the level of Ca^{2+} presented to the mitochondria. The apparent uncoupling became evident at lower concentrations of TCE when in the presence of higher concentrations of Ca^{2+}.

Table 3. Effect of TCE and Mg^{2+} on the distribution of mitochondrial water during State 4 respiration*

Plus 5 mM Mg^{2+}	Control	+ TCE†	Change	% Change
Mitochondrial water	1·532	1·332	−0·200	−13
Sucrose space	0·796	0·279	−0·517	−65
Sucrose-impermeant space	0·736	1·053	+0·317	+52
No Added Mg^{2+}				
Mitochondrial water	1·310	1·299	−0·011	< 1
Sucrose space	0·589	0·743	+0·154	+26
Sucrose-impermeant space	0·721	0·556	−0·165	−24

* Mitochondria were incubated with ^{14}C-sucrose (0·5 μCi/ml) or ^{14}C-inulin (0·5 μCi/ml) and $^{3}H_2O$ (1·0 μCi/ml) in the polarographic reaction mixture (see Methods). After an ADP-induced respiratory control cycle, an aliquot of the mitochondrial suspension was treated as described previously [14]. Data represent the average of duplicate trials obtained from five separate preparations of mitochondria. In all cases, the standard deviation varied ≤ 15% of the indicated value. Mitochondrial volumes are measured in μl/mg of mitochondrial protein.

† TCE concentration tested was 1·0 μmole/mg of mitochondrial protein, sufficient to increase State 4 respiration approximately 2-fold (e.g. see Fig. 3).

However, exposure to TCE lowers the magnitude of H^+ efflux associated with the uptake of added Ca^{2+} (Fig. 7a). The magnitude of this decrease in

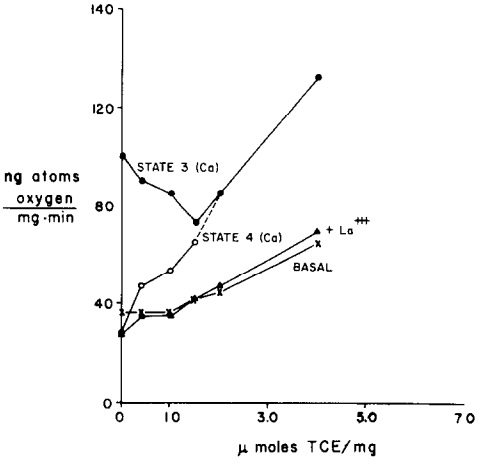

Fig. 6. Effect of TCE on low level Ca^{2+} uptake by rat liver mitochondria. See Methods for experimental details. The data represent the results obtained from one (N = 8) preparation of mitochondria; each point is the average of at least two trials. After 1 min of basal respiration in the presence of varying levels of TCE, State 3 (Ca) respiration was initiated by addition of 100 nmoles Ca^{2+}/mg of mitochondrial protein. After attainment of a stable State 4 (Ca) respiratory trace, 5 nmoles/mg $LaCl_3$ was added and the subsequent alteration of respiration is denoted as + La^{3+}. The dotted line connecting State 4 (Ca) to State 3 (Ca) reflects the apparent release of respiration, i.e. release of respiratory control, between 1·5 and 2·0 μmoles TCE/mg of mitochondrial protein.

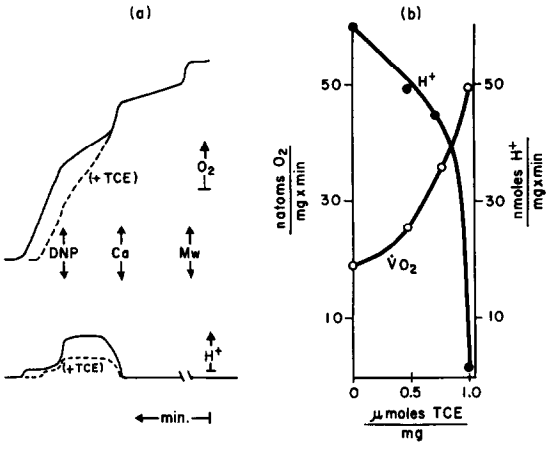

Fig. 7. Effect of TCE on H^+ efflux from rat liver mitochondria associated with low level Ca^{2+} uptake. In part a are shown typical respiratory and H^+ ion changes associated with addition of low levels of Ca^{2+} (50 nmoles/mg of mitochondrial protein) in the presence (dotted line, 1 μmole/mg of mitochondrial protein) or absence of TCE. In part b, the inverse relationship between State 4 (Ca) respiration (VO_2) and H^+ efflux is presented. The data represent the average of duplicate trials from three separate preparations of mitochondria in which 50 nmoles Ca^{2+}/mg of mitochondrial protein was added. Essentially identical results were obtained in seven other preparations of mitochondria in which the dose of Ca^{2+} used to initiate the respiratory cycle was varied. In these cases the crossover point varied toward lower doses of TCE with lower levels of Ca^{2+}.

H^+ efflux is seen to be inversely related to the increased State 4 (Ca) respiratory rate (Fig. 7b).

These data suggest that in the presence of TCE a reduced level of Ca^{2+} (as reflected by the reduced H^+ efflux) is sequestered by the mitochondria, in addition to the apparent increase in energy load [as reflected by the La^{3+}-sensitive State 4 (Ca) respiration]. Alternatively, the data may reflect a TCE-induced leakiness in the mitochondrial membrane such that the ejected H^+ is partially neutralized by admixing with hydroxyl ion generated simultaneously within the mitochondrion's interior [22].

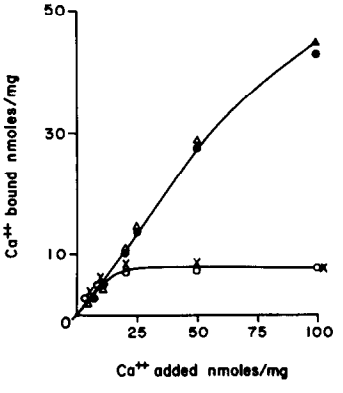

Fig. 8. Effect of 1 μmole TCE/mg of mitochondrial protein on passive and active uptake of Ca^{2+} by rat liver mitochondria. Passive binding studies (lower curve) were carried out at 0° in the presence (×) or absence (○) of TCE. The reaction medium was identical with that used in respiratory studies (see Methods), except that EDTA and exogenous substrate were omitted. The mitochondria were separated from the reaction medium after 10 min of incubation by filtration through a Millipore filter (0·45 μ). Active Ca^{2+} uptake was followed in the oxygen electrode apparatus at 30°. The mitochondria were rapidly harvested, as above, 90 sec after attainment of State 4 (Ca) respiration and $^{45}Ca^{2+}$ uptake was determined (Methods).

In order to clarify the relationship between TCE and Ca^{2+} uptake, H^+ efflux, mitochondrial respiration and mitochondrial permeability properties to Ca^{2+}, passive binding and active uptake were measured with radiolabeled Ca^{2+}. The results of one (N = 8) such experiment are seen in Fig. 8. One μmole TCE/mg of mitochondrial protein had no effect on either the extent of passive binding of Ca^{2+} by the mitochondria or on the magnitude of active uptake, although under these latter conditions State 4 (Ca) respiration is increased 2-fold, as seen in Fig. 6.

From these data it may be argued that the TCE-induced decrement in H^+ efflux (Fig. 7) associated with apparently normal Ca^{2+} uptake (Fig. 8) can be explained by drug-induced leakiness of the mitochondrial membranes, with resultant neutralization of ejected H^+. Further, since the Ca^{2+} binding and uptake capacities are unaltered by TCE, the increased State 4 (Ca) respiration (Fig. 6) is interpreted to reflect an increased energy requirement in order to maintain the sequestered Ca^{2+} bound to mitochondrial binding sites.

ATP-dependent mitochondrial reactions. Mitochondrial ATPase activity, in the absence of exogenous Mg^{2+}, is relatively insensitive to TCE, as seen in Table 4. Neither DNP-stimulated nor oligomycin-insensitive ATPase activity is affected by TCE in concentrations up to 20 μmoles/mg of mitochondrial protein.

In contrast, ATP-driven mitochondrial swelling, associated with the uptake of calcium acetate by the mitochondrion is sensitive to TCE (Fig. 9). When plotted on semi-logarithmic paper (P. Herd, unpublished observation), the response to TCE appears polyphasic and 50 per cent inhibition occurs at 4 μmoles TCE/mg of mitochondrial protein. In view of the relative insensitivity of the mitochondrial ATPase activity to TCE under these conditions (*vide supra*), the data suggest that the primary site of inhibition of the swelling response is on the Ca^{2+} side of the common high energy intermediate, and may therefore be, in part, reflective of the initial inhibition of State 3 (Ca) respiration seen at low doses of TCE (Fig. 6).

Table 4. Effect of 1,1,1-trichloroethane (TCE) on mitochondrial, 2,4-dinitrophenol-sensitive ATPase activity in the absence of exogenous Mg^{2+}

TCE concn. (μmoles/mg)	Activity*	
	+ 2,4-Dinitrophenol	+ Oligomycin
0	0·012 ± 0·001	0·001 ± 0·0004
2	0·012 ± 0·001	0·001 ± 0·0004
5	0·013 ± 0·001	0·001 ± 0·0004
10	0·013 ± 0·001	0·001 ± 0·0003
20	0·012 ± 0·001	0·001 ± 0·0004

* Expressed as μmoles ATP hydrolyzed/mg × min and obtained from freshly prepared mitochondria (N = 5), as described in Methods.

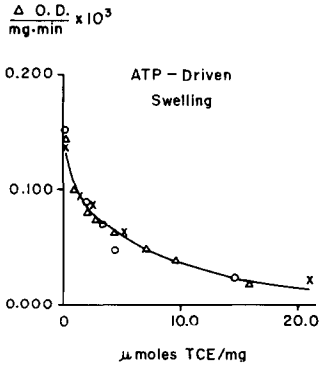

Fig. 9. Effect of TCE on ATP-induced mitochondrial swelling associated with the uptake of calcium acetate. See Methods for experimental details. The data represent the average of duplicate trials obtained from three preparations of mitochondria. The curve is drawn as a best fit by eye.

DISCUSSION

The toxic effects of halocarbons have long been recognized [23]; however, except for a few limited cases, e.g. halothane [24–26] and carbon tetrachloride [27, 28], the molecular bases for their toxicity have not been explored, except in general terms of anesthetic action [29]. In view of the lipophilicity of these compounds [23], it is assumed that halocarbons have a high affinity for biological membranes, which thus might be expected to be a primary site of action [17].

In the case of TCE, although it is not appreciably catabolized after inhalation [30], it, like other halocarbons [31], is expected to have a high tissue/blood partition coefficient; this view is supported by the concentration-dependent retention of the drug long after exposure [1, 32].

One purpose of these studies was an attempt to ascertain the basis for the depression of myocardial contractility induced by TCE [8], but protected and/or relieved by exogenous Ca^{2+}. These latter observations suggested that the Ca^{2+}-sensitive contractile apparatus was intact under conditions of acute exposure and it was proposed that: (a) an inhibition of Ca^{2+} release, (b) decreased sensitivity to endogenous levels of Ca^{2+}, as recently suggested by Ohnishi *et al.* [33] in studies on halothane, and/or (c) lack of adequate energy to mediate the contractile process may be involved in this depression of contractility. Study of the handling of Ca^{2+} by the contractile apparatus as well as the actual tissue/blood partition coefficient for TCE is currently under investigation.*

That the supply of conserved energy may be the factor limiting myocardial contractile behavior under conditions of acute exposure was derived from the observation of Krantz *et al.* [7] that TCE inhibits the respiration of myocardial tissue slices; these data are supported by our observations on the sensitivity of the isolated mitochondria to TCE. These subcellular actions of TCE are in many respects similar and within one order of magnitude more toxic than those of halothane [25],* and at least one order of magnitude less toxic than that reported for carbon tetrachloride [27, 28], as evidenced from comparable studies on the lability of the rotenone-sensitive site on the electron transport chain [24, 25, 27] and the appearance (release) of an ATPase activity dependent on exogenous Mg^{2+} [26].

The effect of halocarbons on the response of mitochondria to low levels of Ca^{2+} has not been well studied, except for the recent report of Miller and Hunter [34]. These investigators demonstrated a halothane-induced decrease in the rate of Ca^{2+} uptake by rat liver mitochondria, but the amount of Ca^{2+} sequestered remained unchanged, and further inspection of their data on the release of bound Ca^{2+}, induced by dinitrophenol, indicates that the rate of release is enhanced with increasing levels of halothane. These data are consistent with and extended by our observations that: (a) the rate of Ca^{2+} uptake [as measured by State 3 (Ca)] is initially depressed by TCE (Fig. 6); (b) the levels of Ca^{2+} passively bound and actively sequestered are unaffected by TCE (Fig. 8); and (c) the permeability properties of the mitochondria are altered, resulting in a continuous, energy-dissipating, passive efflux and active re-uptake of the added Ca^{2+}, as evidenced by the increased State 4 (Ca) oxygen consumption with increasing levels of the drug (Fig. 6).

Thus, besides emphasizing the similarities of action between halothane and TCE, these data suggest that in addition to a decreased capacity to supply reducing equivalents for energy conservation (and hence ATP synthesis), the halocarbon causes the release of at least two additional energy-dissipating processes: (a) an Mg^{2+}-dependent ATPase activity (Fig. 5, Table 2), and (b) an alteration in mitochondrial membrane permeability, at least with respect to Ca^{2+} and H^+ (Figs. 6 and 7). Although it is not possible at the present time to extrapolate these observations *in vitro* to the situation of acute exposure to the drug *in vivo*, we may speculate that the protective effect of exogenous Ca^{2+} on myocardial contractility seen during exposure to TCE [8] may result from optimization of the responsiveness of the contractile mechanism under conditions of reduced energy supply. Additional studies are indicated before definitive conclusions can be made with regard to this hypothesis or the related sequelae associated with halocarbon-induced depression of myocardial contractility [33].* However, the present experiments provide a mechanistic basis for the depression of mitochondrial metabolism [7] during acute exposure to TCE.

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