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INTERACTIONS OF ACETALDEHYDE, ETHYL ALCOHOL AND OXYBARBITURATES AFFECTING MITOCHONDRIAL FUNCTIONS*

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Abstract—The effects of combinations of acetaldehyde, ethyl alcohol and an oxybarbiturate on the rate of oxygen consumption, the ADP/O ratio and energy-dependent swelling by rat liver mitochondria have been investigated. The effects of similar combinations on the rate of oxygen uptake by submitochondrial preparations, oxidizing several different substrates, have also been studied. Acetaldehyde at millimolar levels increased the rate of oxygen consumption by rat liver mitochondria which had been preincubated with ADP, inhibited NAD^+-linked oxidations by mitochondrial and submitochondrial preparations, and increased the rate of the energy-dependent swelling of rat liver mitochondria with succinate as substrate. Combinations of acetaldehyde, ethyl alcohol and an oxybarbiturate interacted to reduce the rate of oxygen consumption by rat liver mitochondria and the energy-dependent swelling of rat liver mitochondria when DL-3-hydroxybutyrate was the substrate. Ethyl alcohol was not involved in these interactions. The effects of all combinations tested could be ascribed to interaction(s) between acetaldehyde and an oxybarbiturate or to activities of acetaldehyde or the oxybarbiturate only. In the case of state 3 respiration and uncoupled mitochondrial respiration, acetaldehyde and the tested oxybarbiturates behaved as additive inhibitors. All of the results are consistent with the view that the NADH-ubiquinone segment (Complex 1) of the mitochondrial electron transport chain is the site of an acetaldehyde-oxybarbiturate interaction to inhibit electron transport.

IT IS WELL established that ethanol and an oxybarbiturate can interact *in vivo*;[1,2] however, this interaction has not been extensively investigated *in vitro*. Known activities of acetaldehyde, a major metabolite of ethanol, and the oxybarbiturates suggested that these substances could interact to alter mitochondrial functions. The oxybarbiturates are inhibitors of electron transport by the NADH–ubiquinone segment (Complex 1) of the mitochondrial electron transport chain.[3–5] The oxybarbiturates also have some activity as oligomycin-like inhibitors of energy transport.[6,7] Acetaldehyde has been reported to have the following effects on mitochondrial preparations: inhibition of pyruvate oxidation;[8–10] lowering of the concentration of coenzyme A;[11] and stimulation of energy-dependent swelling.[12] In addition, several investigators have shown that acetaldehyde is slowly oxidized by mitochondrial preparations.[12–15]

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The primary purpose of this paper is to report and discuss the results of experiments designed to detect and describe interactions between acetaldehyde, ethanol and oxybarbiturates which alter the function of isolated rat liver mitochondria.

MATERIALS AND METHODS

Chemicals. The CCP* was a gift from the E. I. DuPont Nemours Company. Oxybarbiturates were from the Eli Lilly Company and the Sigma Chemical Company. Acetaldehyde was from the Eastman Kodak Company; all other reagents were the best grades available from the Sigma Chemical Company or the Fisher Scientific Company. Physical properties of the acetaldehyde were as follows: boiling range 20–22°; specific gravity 20/4 0·783 ± 0·003; index of refraction r 20/d 1·3325 ± 0·0010. In the ultra-violet region the acetaldehyde, diluted with water, absorbed weakly between 240 and 300 nm; the maximum molar extinction coefficient observed was 7·4 at 278 nm. In the infrared region, the frequencies of all absorption maxima of the undiluted acetaldehyde corresponded well with those of published, infrared acetaldehyde specta. The acetaldehyde preparation was also analyzed by gas–liquid chromatography using flame ionization detection and a 2 m × 4 mm column of 3% OV-101 coated on 80/100 mesh H.P. Chromosorb W. The gas chromatogram of the acetaldehyde preparation consisted of one major peak and a very minor peak, present at a level near the limit of detection, which had a retention time greater than the major component. The acetaldehyde was removed from the ampule in which it was received and stored at 0–2° in a capped bottle. Aqueous solutions of acetaldehyde were prepared by dilution using 0·780 as the specific gravity of the cold acetaldehyde. Acetaldehyde and sodium oxybarbiturate solutions were prepared fresh daily.

Analytical methods. The protein concentration of mitochondrial preparations was determined by the biuret method[16] with bovine serum albumin as the protein standard. Sodium deoxycholate at a final concentration of 0·33% was added to the biuret assay to solubilize particulate matter. Sodium ADP solutions were standardized by the enzymic method described by Miller and Hunter[17] and/or by extinction at 260 nm using 15·4 as the millimolar extinction coefficient for ADP.

Mitochondrial preparations. Mitochondria were isolated from the livers of 200–250 g, male rats of the Sprague–Dawley strain. The animals were maintained on Purina Lab Chow and water *ad lib.* Experiments with intact liver mitochondria were completed within 5 hr of the completion of the isolation procedure.

For all studies in which the consumption of oxygen was followed, mitochondria were isolated as described by Johnson and Lardy[18] with the only modification being that before the initial homogenization the tissue was processed with a stainless steel tissue press having 1 mm openings. The final mitochondrial pellet was suspended in the isolation medium at a protein concentration of 60 mg/ml.

Liver mitochondria for studies of energy-dependent swelling were isolated as described by Miller and Hunter[17] with preliminary processing of the tissue with the tissue press as described above. The final mitochondrial pellet was suspended in 0·33 M sucrose at a protein concentration of 40–50 mg/ml. A more dilute suspension was prepared from the stock mitochondrial suspension just before starting an experiment.

* Unusual abbreviations used are as follows: carbonyl cyanide *m*-chlorophenylhydrazone, CCP; submitochondrial particles prepared from beef heart mitochondria, ETP.

The ETP preparations used in this study, prepared by the method of MacLennan *et al.*,[19] were a gift from Dr. R. A. Harris of the Department of Biochemistry of the Indiana University Medical Center.

Determination of the rate of oxygen consumption. Oxygen consumption was followed with a Gilson Oxygraph fitted with a 4004 Clark Oxygen Probe. The Oxygraph cell, which was nearly filled by the 1·5-ml reaction mixture, was open to the atmosphere. The temperature of the cell was regulated at 30 ± 0·5°. The contents of the cell were stirred with a magnetic bar rotated at a speed just below that which would have caused the surface of the reaction mixture to vortex. The solubility of oxygen in the standard respiration medium (0·25 M sucrose, 10 mM potassium phosphate, pH 7·45, 2 mM magnesium chloride) was 489 n-atoms/ml at 30° as determined by the method of Estabrook and Mackler.[20]

Procedure for determination of the ADP/O and rate of respiration with intact mitochondria. The complete suspension (1·5 ml) contained 375 μmoles of sucrose, 15 μmoles of potassium phosphate, pH 7·45, 3·0 μmoles of magnesium chloride, the indicated final concentrations of test substances, 4·5 mg of mitochondrial protein, 7·5 μmoles of the sodium salt of the indicated oxidizable substrate, 300 nmoles of ADP (150 nmoles of ADP when succinate was the substrate), and 0·75 nmole of CCP. The mitochondrial suspension (75 μl), substrate (20 μl), ADP (50 μl), and CCP (15 μl) were sequentially added to the other components in the Oxygraph cell so that the rate of oxygen uptake under state 3 and state 4 conditions and the ADP/O could be calculated as illustrated by Estabrook.[21] The CCP was added after the rate of state 4 respiration had been recorded for 2 min. The maximum rate of respiration obtained after the addition of CCP was defined as uncoupled.

Procedure for observation of energy-dependent, phosphate-induced swelling of liver mitochondria. Optical measurements were made with a Beckman DU-2 spectrophotometer using rectangular, glass cuvettes with a 1·0 cm light path and a volume of 3·5 ml. These experiments were conducted at 24 ± 1°. The procedure was similar to that described by Miller and Hunter.[17] The complete suspension (3·0 ml) contained 990 μmoles of sucrose, 150 μmoles of Tris hydrochloride at pH 7·45, 30 μmoles of potassium phosphate at pH 7·45, 12 μmoles of the sodium salt of the indicated substrate, the indicated test substances and 450 μg of mitochondrial protein. The reaction was started with the addition of the mitochondria. The first reading of the extinction of the suspension was taken about 1 min after the addition of the mitochondria with the spectrophotometer adjusted so that the initial extinction reading was 0·500 ± 0·050 at 520 nm.

RESULTS

Effects of acetaldehyde on the respiration of mitochondrial and submitochondrial preparations. Acetaldhyde stimulated the rate of oxygen uptake of a suspension of mitochondria after preincubation with ADP to reduce the level of endogenous substrates (Table 1). The maximal rate of oxygen uptake after the addition of 2·5 to 10 mM acetaldehyde was about 25 per cent of the rate of state 3 respiration with DL-3-hydroxybutyrate as substrate (see Tables 3–5). Twenty mM acetaldehyde completely inhibited oxygen uptake.

Preliminary experiments demonstrated that acetaldehyde could also inhibit the rate of oxygen uptake by intact mitochondria in the presence of substrates which are

TABLE 1. OXYGEN CONSUMPTION BY MITOCHONDRIA IN THE PRESENCE OF ACETALDEHYDE*

Acetaldehyde (mM)	Oxygen uptake (n-atoms/min and mg of protein)		
	Before acetaldehyde	After acetaldehyde	After rotenone
2·5	2·7	16·6	0·0
5·0	2·5	16·6	0·0
10·0	2·7	16·6	0·0
20·0	2·7	00·0	0·0

* The assay (1·5 ml) contained 375 μmoles of sucrose, 15 μmoles of potassium phosphate at pH 7·45, 3·0 μmoles of magnesium chloride, the indicated concentration of acetaldehyde, 325 nmoles of ADP and 4·5 mg of mitochondrial protein. The mixture, complete except for acetaldehyde, was completed and incubated for 3 min. The rate of oxygen uptake at the end of 3 min is recorded in the "Before acetaldehyde" column. The acetaldehyde was added after the preincubation with ADP. The "After acetaldehyde" rate of oxygen uptake recorded was observed 3 min after the addition of acetaldehyde. Finally, 3 μg of rotenone dissolved in 5 μl of 50% (v/v) dimethylsulfoxide was added. The addition of rotenone immediately inhibited oxygen uptake.

oxidized by NAD^+-dependent pathways. Table 2 shows that acetaldehyde also inhibits the oxidation of NADH by two submitochondrial preparations which are capable of rapidly oxidizing exogenous NADH. However, respiration by these preparations is

TABLE 2. EFFECT OF ACETALDEHYDE ON THE RATE OF OXYGEN UPTAKE BY CHOLATE-TREATED MITOCHONDRIA OR ETP WITH NADH OR SUCCINATE AS SUBSTRATE*

Acetaldehyde (mM)	Rate of oxygen uptake (n-atoms/min and mg protein)			
	Cholate-treated mitochondria		ETP	
	Substrate		Substrate	
	NADH	Succinate	NADH	Succinate
0·0	139	110	565	365
0·5	114	110	548	374
1·0	100	114	532	349
2·5	98	105	399	366
5·0	69	116	316	349
10·0	50	105	100	366
15·0	42	103	83	366
50·0	28	105	67	332

* The following were placed in the Oxygraph cell in a volume of 1·4 ml: 375 μmoles of sucrose, 15 μmoles of potassium phosphate, pH 7·45, 3 μmoles of magnesium chloride, the indicated concentration of acetaldehyde and 4·5 mg of mitochondrial protein. The recording of oxygen consumption was started and then 70 μl of 10% (w/v) cholic acid (titrated to pH 7·5 with sodium hydroxide) was added. One min after the addition of cholate, 50 μl of 17 mM NADH or 20 μl of 0·5 M sodium succinate was added. The rate of oxygen uptake was calculated from the linear portion of the Oxygraph trace obtained after the addition of succinate or NADH. The procedure was the same when the rate of oxygen uptake by ETP was studied except that no cholate was added and the final ETP protein concentration was 0·50 mg/ml.

not inhibited by 1–10 mM acetaldehyde when succinate is the substrate. These results are consistent with earlier reports which demonstrated that intact mitochondria can oxidize acetaldehyde.[12–15] The results also suggest that acetaldehyde is capable of inhibiting electron transport by the NADH-ubiquinone segment (Complex 1) of the mitochondrial electron transport chain.

Effects of combinations of acetaldehyde, ethyl alcohol and an oxybarbiturate on mitochondrial respiration and oxidative phosphorylation. Since it was of interest to determine the nature of any interactions among the agents, a factorial experiment in which a complete randomized design was applied, with each agent at two dose levels (2^3), was the simplest approach.[22] Tables 3–6 contain the data from experiments using this design with DL-3-hydroxybutyrate or succinate as substrate. The level of acetaldehyde and oxybarbiturate used in these experiments was sufficient to produce a 30–50 per cent inhibition in the rate of state 3 respiration with DL-3-hydroxybutyrate as substrate as determined in preliminary experiments. Forty mM ethanol was chosen to approximate the level found in blood after heavy drinking since preliminary experiments demonstrated that ethanol alone, at concentrations less than 200 mM, had no detectable effect on the measured parameters.

To analyze the data contained in Tables 3–5, the null hypothesis was made that the interaction was additive. The null hypothesis was accepted if the P value was greater than 0·05; otherwise it was rejected. Conventional analysis of variance for the main effect and the interaction was accomplished with the aid of a computer. Analysis of the data (Tables 3–5) showed that the main effects of acetaldehyde and the oxybarbiturates on the rate of respiration with DL-3-hydroxybutyrate as substrate were significant with P values less than 0·005. The null hypothesis that the interaction was additive was accepted for the parameters, state 3 and uncoupled respiration, with all P values being greater than 0·05 (Tables 3–5). Thus, the data supported the view that acetaldehyde and the tested oxybarbiturates behaved as additive inhibitors of state 3 and uncoupled respiration when DL-3-hydroxybutyrate was the substrate. For the parameters ADP/O and state 4 respiration, the interaction between acetaldehyde and secobarbital or phenobarbital was statistically significant; thus, for these parameters, the combined effect of acetaldehyde and secobarbital or phenobarbital was more than additive. However, the null hypothesis that the interaction of acetaldehyde with amobarbital was additive in nature for the inhibition of the ADP/O and state 4 respiration was accepted with all P values being greater than 0·05. Analysis of the data in Tables 3–5 showed that ethanol, at the level tested, was not involved in any of the interactions.

Table 6 contains the data from the investigation of the effects of combinations of acetaldehyde, ethanol and amobarbital with succinate as substrate. Analysis of these data as outlined above demonstrated that there was no interaction between acetaldehyde, ethanol and amobarbital when succinate was the substrate with all P values being greater than 0·25. Experiments identical to that recorded in Table 6 were completed with 125 μM secobarbital or 2 mM phenobarbital replacing 125 μM amobarbital. Again, no evidence of interaction was obtained. Thus with succinate as substrate, there was no interaction between acetaldehyde, ethanol and the tested oxybarbiturates to affect the rate of respiration or ADP/O.

Effects of combinations of acetaldehyde and secobarbital on mitochondrial respiration. Inspection of Fig. 1 shows that, in the case of intact mitochondria oxidizing

TABLE 3. EFFECTS OF COMBINATIONS OF ACETALDEHYDE, ETHYL ALCOHOL AND SECOBARBITAL ON MITOCHONDRIAL RESPIRATION AND OXIDATIVE PHOSPHORYLATION WITH DL-3-HYDROXYBUTYRATE AS SUBSTRATE*

Treatment			Rate of oxygen uptake (n-atoms/min and mg protein)			
Secobarbital (125 μM)	Acetaldehyde (5 mM)	Ethanol (40 mM)	State 3	State 4	Uncoupled	ADP/O
−	−	−	60·8 ± 2·83	12·4 ± 0·12	79·9 ± 4·62	2·02 ± 0·10
+	−	−	40·9 ± 2·66	10·9 ± 0·42	61·2 ± 3·82	2·01 ± 0·12
−	+	−	37·7 ± 2·26	10·6 ± 0·57	54·9 ± 3·14	1·90 ± 0·07
−	−	+	64·1 ± 3·48	13·1 ± 0·32	87·5 ± 8·90	2·12 ± 0·16
+	−	+	40·1 ± 3·36	10·1 ± 0·35	62·5 ± 4·90	2·04 ± 0·03
−	+	+	39·8 ± 2·59	10·2 ± 0·59	56·3 ± 4·86	1·93 ± 0·05
+	+	−	14·4 ± 0·69	6·9 ± 0·29	35·1 ± 1·79	1·54 ± 0·04
+	+	+	14·0 ± 0·90	6·8 ± 0·19	35·6 ± 2·49	1·57 ± 0·05

* The procedure was as described in the Materials and Methods section. The values recorded represent the mean ± the standard error for three replications with three different mitochondrial preparations.

TABLE 4. EFFECTS OF COMBINATIONS OF ACETALDEHYDE, ETHYL ALCOHOL AND AMOBARBITAL ON MITOCHONDRIAL RESPIRATION AND OXIDATIVE PHOSPHORYLATION WITH DL-3-HYDROXYBUTYRATE AS SUBSTRATE*

Treatment			Rate of oxygen uptake (n-atoms/min and mg protein)			
Amobarbital (125 μM)	Acetaldehyde (5 mM)	Ethanol (40 mM)	State 3	State 4	Uncoupled	ADP/O
−	−	−	80·0 ± 2·19	15·2 ± 0·78	101·6 ± 2·30	2·31 ± 0·03
+	−	−	67·4 ± 0·93	15·2 ± 0·78	89·5 ± 0·94	2·20 ± 0·10
−	+	−	50·0 ± 2·77	13·4 ± 1·21	73·5 ± 2·93	2·18 ± 0·12
−	−	+	80·8 ± 3·20	14·8 ± 0·90	107·1 ± 0·90	2·25 ± 0·08
+	−	+	66·0 ± 2·30	14·3 ± 0·43	90·5 ± 3·34	2·28 ± 0·13
−	+	+	48·9 ± 3·33	13·4 ± 0·92	74·9 ± 3·18	2·16 ± 0·12
+	+	−	30·0 ± 1·66	11·1 ± 0·01	59·1 ± 3·70	1·95 ± 0·08
+	+	+	27·2 ± 0·50	10·6 ± 0·47	61·9 ± 3·33	1·99 ± 0·16

* The procedure was as described in the Materials and Methods section. The values recorded represent the mean ± the standard error for three replications with three different mitochondrial preparations.

TABLE 5. EFFECTS OF COMBINATIONS OF ACETALDEHYDE, ETHYL ALCOHOL AND PHENOBARBITAL ON MITOCHONDRIAL RESPIRATION AND OXIDATIVE PHOSPHORYLATION WITH DL-3-HYDROXYBUTYRATE AS SUBSTRATE*

Treatment			Rate of oxygen uptake (n-atoms/min and mg protein)			
Phenobarbital (2 mM)	Acetaldehyde (5 mM)	Ethanol (40 mM)	State 3	State 4	Uncoupled	ADP/O
−	−	−	85·7 ± 2·69	15·5 ± 0·93	102·4 ± 6·36	2·23 ± 0·09
+	−	−	61·2 ± 4·83	13·9 ± 1·37	74·4 ± 4·81	2·26 ± 0·11
−	+	−	51·7 ± 3·70	13·0 ± 0·44	74·9 ± 4·79	2·17 ± 0·16
−	−	+	85·8 ± 2·77	14·3 ± 0·43	101·6 ± 5·62	2·30 ± 0·14
+	−	+	55·4 ± 4·79	15·3 ± 1·37	77·6 ± 4·21	2·25 ± 0·14
−	+	+	56·3 ± 6·68	13·7 ± 1·77	70·3 ± 2·50	2·21 ± 0·10
+	+	−	18·0 ± 2·88	8·8 ± 0·47	48·0 ± 4·88	1·76 ± 0·06
+	+	+	18·0 ± 3·65	9·2 ± 0·93	48·0 ± 5·62	1·74 ± 0·06

* The procedure was as described in the Materials and Methods section. The values recorded represent the mean ± the standard error for three replications with three different mitochondrial preparations.

TABLE 6. LACK OF EFFECT OF COMBINATIONS OF ACETALDEHYDE, ETHYL ALCOHOL AND AMOBARBITAL ON MITOCHONDRIAL RESPIRATION AND OXIDATIVE PHOSPHORYLATION WITH SUCCINATE AS SUBSTRATE*

Treatment			Rate of oxygen uptake (n-atoms/min and mg protein)			
Amobarbital (125 μM)	Acetaldehyde (5 mM)	Ethanol (40 mM)	State 3	State 4	Uncoupled	ADP/O
−	−	−	141·6 ± 7·16	43·5 ± 1·52	250·9 ± 10·33	1·45 ± 0·04
+	−	−	145·9 ± 7·58	46·2 ± 2·44	261·3 ± 14·41	1·42 ± 0·05
−	+	−	134·3 ± 9·01	43·9 ± 1·66	263·2 ± 11·17	1·39 ± 0·03
−	−	+	141·3 ± 8·30	45·2 ± 0·93	254·8 ± 13·83	1·48 ± 0·02
+	−	+	146·8 ± 7·32	45·7 ± 0·81	267·8 ± 8·80	1·37 ± 0·06
−	+	+	138·5 ± 6·41	44·8 ± 1·24	257·6 ± 8·89	1·38 ± 0·07
+	+	−	142·2 ± 4·88	46·6 ± 1·21	249·3 ± 8·46	1·41 ± 0·02
+	+	+	143·1 ± 5·62	44·3 ± 1·59	252·1 ± 12·68	1·35 ± 0·02

* The procedure was as described in the Materials and Methods section. The values recorded represent the mean ± the standard error for three replications with three different mitochondrial preparations.

DL-3-hydroxybutyrate, 0·5 to 10 mM acetaldehyde and 20 μM secobarbital behaved as additive inhibitors of oxygen uptake as did 0·5 to 5 mM acetaldehyde in combination with 125 μM secobarbital.

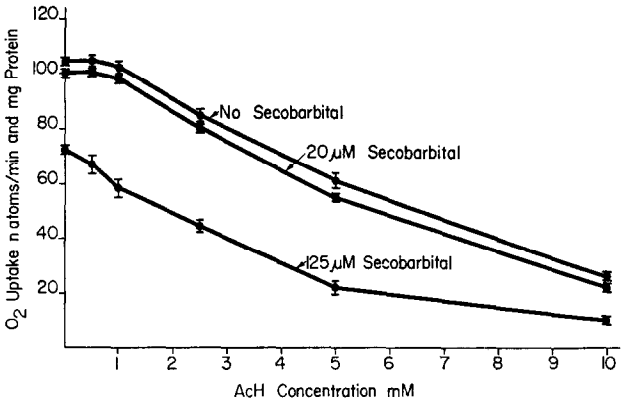

FIG. 1. Inhibition by combinations of acetaldehyde and secobarbital of mitochondrial, state 3 respiration with DL-3-hydroxybutyrate as substrate. The procedure was as described in the legend of Table 3 except that the final concentrations of acetaldehyde and secobarbital were varied as indicated. The points in the figure represent the mean $\pm$ the standard error for three replications with three different mitochondrial preparations.

Figure 2 illustrates that combinations of 0·5–5 mM acetaldehyde with 20 or 125 μM secobarbital also produced additive inhibition of the rate of oxidation of NADH by ETP. The same actaldehyde–secobarbital combinations had no effect on the rate of succinate oxidation by ETP or intact mitochondria.

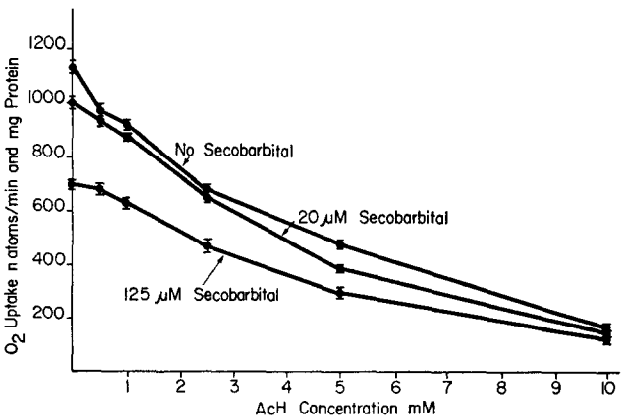

FIG. 2. Inhibition by combinations of acetaldehyde and secobarbital of the oxidation of NADH by ETP. The procedure was as described for determination of the rate of NADH oxidation by ETP in the legend of Table 2 except the final concentrations of acetaldehyde and secobarbital were varied as indicated. The points represent the mean $\pm$ the standard error for three replications with the same ETP preparation.

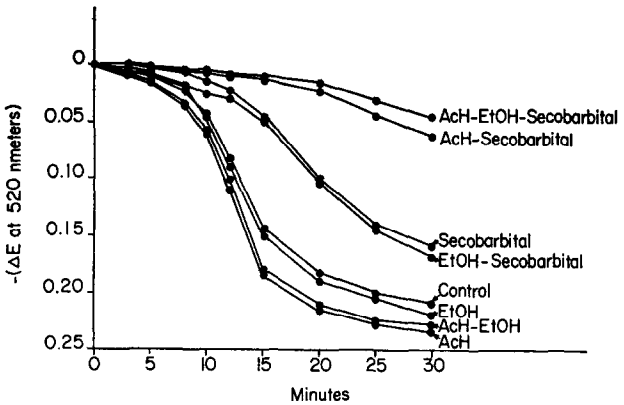

FIG. 3. Effect of combinations of acetaldehyde, ethyl alcohol and secobarbital on energy-dependent, phosphate-induced mitochondrial swelling with DL-3-hydroxybutyrate as substrate. The procedure was as described in the Materials and Methods section.

Interactions of acetaldehyde, ethyl alcohol and secobarbital affecting energy-dependent, phosphate-induced swelling of mitochondria. Energy-dependent, phosphate-induced mitochondrial swelling is thought to result from the energy-dependent[23] uptake of ions accompanied by water sufficient to maintain osmotic neutrality between the space enclosed by the mitochondrial inner membrane and the medium.[24] Byington and Yeh[12] have recently reported that acetaldehyde is capable of increasing the rate and extent of the swelling of rat liver mitochondria suspended in a potassium chloride–Tris hydrochloride medium. Preliminary experiments showed that acetaldehyde produced

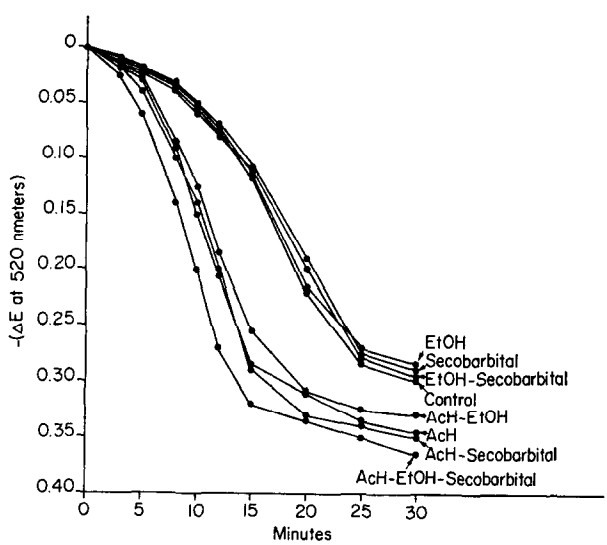

FIG. 4. Effect of combinations of acetaldehyde, ethyl alcohol and secobarbital on energy-dependent, phosphate-induced mitochondrial swelling with succinate as substrate. The procedure was as described in the Materials and Methods section.

qualitatively similar changes in the rate and extent of the energy-dependent, phosphate-induced swelling of mitochondria suspended in the sucrose medium used in this investigation.

The data reported in Fig. 3 are typical of the results of the investigation of the effects of acetaldehyde–ethanol–secobarbital combinations on mitochondrial swelling with DL-3-hydroxybutyrate as substrate. Acetaldehyde–secobarbital combinations inhibited swelling; however, since the changes in optical properties of the suspensions are probably only qualititavely related to the degree of swelling,[25] the nature of this interaction cannot be determined from these data.

Figure 4 shows the effects of the same combination of agents on mitochondrial swelling with succinate as the exogenous substrate. Any treatment which included 5 mM acetaldehyde stimulated the rate and extent of mitochondrial swelling. None of the treatements inhibited swelling. This is in agreement with the results obtained above which indicated that acetaldehyde and secobarbital did not interact to inhibit mitochondrial respiration when succinate was the substrate; therefore, only the stimulation of swelling by acetaldehyde was observed.

DISCUSSION

The results clearly establish that, under the experimental conditions employed, ethyl alcohol did not ineract with acetaldehyde or the oxybarbiturates to alter the measured mitochondrial functions. On the other hand, all of the observed effects of the combinations appeared to be due to interaction between acetaldehyde and an oxybarbiturate.

Several lines of evidence served to identify the NADH-ubiquinone segment (Complex 1) of the mitochondrial electron transfer chain as the site of an acetaldehyde–oxybarbiturate interaction to inhibit respiration. First, acetaldehyde, as well as the oxybarbiturates, alone was capable of inhibiting mitochondrial NAD^+-linked oxidations. Second, acetaldehyde–oxybarbiturate combinations, which inhibited NAD^+-linked oxidations by mitochondrial and submitochondrial pereparations in an additive manner, did not inhibit respiration by the same preparations when succinate was the substrate. Thus the portion of the electron transport chain most sensitive to inhibition by these combinations appeared to be on the substrate side of the point where electrons from succinate enter the electron transport chain. Third, the acetaldehyde–ethyl alcohol–oxybarbiturate interaction was additive in nature to inhibit both state 3 and and uncoupled respiration by intact mitochondria with DL-3-hydroxybutyrate as substrate. This observation suggested that the effects of acetaldehyde–ethyl alcohol–oxybarbiturate combinations on the kinetics of oxygen consumption were due to effects on electron transport and not due to effects on the combinations on the kinetics of energy transport. Finally, the observation that acetaldehyde–ethyl alcohol–secobarbital combinations interacted to inhibit energy-dependent mitochondrial swelling only when DL-3-hydroxybutyrate was substrate was consistent with the view that electron transport at the NADH-ubiquinone segment (Complex 1) of the electron transport chain was a site of an acetaldehyde–oxybarbiturate interaction.

Analysis of the data (Tables 3–5) showed that acetaldehyde–oxybarbiturate combinations reduced the ADP/O ratio. No reduction in the ADP/O was observed when succinate was the substrate (Table 6). Rather than an interaction to produce uncoupling of oxidative phosphorylation, an activity which acetaldehyde and the oxybarbiturates alone did not have at the levels present in the combination studies, it is probable

that the observed reduction in the ADP/O ratio was due to a reduction in coupling efficiency as the flux of electrons through the electron transport chain was reduced due to the interaction to inhibit respiration by the NADH-ubiquinone segment (Complex 1) of the electron transport chain. This interpretation is consistent with a previous study which indicated that coupling efficiency is reduced during slow respiration.[26]

The results of this investigation suggest that a potential exists *in vivo* for acetaldehyde, generated by the oxidation of ethyl alcohol, to alter mitochondrial functions either when present alone or in combination with drugs, such as the oxybarbiturates, which are also capable of inhibiting electron transport at the NADH-ubiquinone segment (Complex 1). Finally the important role of acetaldehyde in the effects of combinations studied here suggests a rational basis for the application of drugs which specifically inhibit alcohol dehydrogenase activity *in vivo*[27] in the clinical management of intoxication by ethyl alcohol–oxybarbiturate combinations.

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