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THE MITOCHONDRIAL RESPIRATORY CONTROL AND OXIDATIVE PHOSPHORYLATION OF ETHANOL FED RATS

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ABSTRACT

Male albino (Wistar strain) rats were fed with ordinary laboratory chew, Oriental pellet, properly balanced with regard to protein, lipids, carbohydrates, minerals, and vitamins, and with free water supply for ten days. Thereafter, the rats were force-fed daily with 4.5 ml of 30% ethanol solution or glucose solution, isocaloric with the former, by a gastric tube for another two weeks. The respiratory control index and AMP/O ratio, the parameters which represent the intactness of mitochondria and the efficiency of the oxidative phosphorylation of liver mitochondria, were measured with liver mitochondria of the control and the alcohol-fed rats. Both indexes were found to be significantly lowered in the alcohol-fed rats indicating that ethanol feeding causes partial uncoupling of the oxidative phosphorylation in the liver mitochondria. The addition of bovine serum albumin *in vitro* did not recover this partial uncoupling. In the alcohol-fed rats for two weeks, the concentration of NEFA, ethanol, and acetaldehyde in the serum were increased which suggests that the increase has close relation with the disturbance of mitochondrial functions in alcohol fed rats, as NEFA is one of the well-known uncouplers. In order to elucidate the effects of alcohol and acetaldehyde on the mitochondrial oxidative phosphorylation *in vitro*, different amounts of alcohol or acetaldehyde were added to the mitochondrial suspension of the control group. Addition of more than 40 μmoles of ethanol/ml of medium or more than 10 μmoles of acetaldehyde/ml of medium impaired both the respiratory control index and AMP/O ratio.

It is noted that the minimum concentration of ethanol effective for the isolated mitochondrial respiratory control and AMP/O ratio was 40 μmoles/ml, the concentration which could be observed in the serum of the alcohol fed rats *in vivo*, but that of acetaldehyde was too high to be observed in ethanol fed rats *in vivo*.

INTRODUCTION

Many researchers[1)-4)] have reported the pathogenesis of alcoholic liver

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damage, but its precise mechanism has not been clearly elucidated. Kiessling and Pilström[5] have observed through electron-micrographs an enlargement of mitochondria in the livers of alcohol-treated rats. Beer and Quastel have shown that acetaldehyde inhibits the respiration of rat brain mitochondria and brain cortex slices[6]. Alcohol added to rat liver slices has been shown to depress the activity of the citric acid cycle[7]. Brodie *et al.*[2] and Katsumata and Yamada[4] have observed increased level of serum NEFA in alcohol-toxicated rats. Long chain unsaturated fatty acid is one of the well-known uncouplers in oxidative phosphorylation. These reports suggest that habitual alcohol drinking may cause changes in the mitochondrial functions. There are few papers dealing with ethanol and function of mitochondria. In these connection, we studied mitochondrial functions of alcohol fed rats and the effect of alcohol and acetaldehyde to normal rat liver mitochondria by measuring AMP/O and the respiratory control index (R.C.I.), parameters which represent the intactness of mitochondria and the efficiency of oxidative phosphorylation.

MATERIALS AND PROCEDURE

Twenty Wistar male rats (body weight of around 100 g) were fed an Oriental laboratory pellet with free water supply for 10 days in a temperature controlled room. Six of the rats were then force-fed daily with 4.5 ml of ethanol solution (30% v/v) by gastric tube, and the other fourteen rats were force-fed with glucose solution of the equivalent calorie to the alcohol solution in addition to the ordinary diet. The former were designated as the alcohol-fed group, the latter the control group. Body weight of the rats was measured daily. After two weeks with alcohol or glucose solution force-fed, six rats of each groups were killed by decapitation after 5 hours of fasting, and the liver was quickly removed and homogenized in a polytron homogenizer. Liver mitochondria were prepared according to the method of Chance and Hagihara[8] (See Table 1). The oxygen uptake of mitochondria was measured by using a Beckman's polarographic oxygen electrode (Clark-type) and a potentiometric recorder, with or without an addition of bovine serum albumin. Respiratory control index (R.C.I.) and AMP/O ratio were calculated by the method of Chance and Williams[9],

$$\text{AMP/O} = \mu\text{moles of AMP added}/\mu \text{ atoms of oxygen consumption}$$

$$\text{R.C.I.} = \frac{\text{Rate of oxygen consumption with AMP}}{\text{Rate of oxygen consumption without AMP}} = \frac{\text{Rate of state 3 respiration}}{\text{Rate of state 4 respiration}}$$

The oxygen uptake of mitochondria of the another eight rats in the control group was also measured after adding various concentrations of ethanol or

TABLE 1. Preparation of Mitochondria

3 g of rat liver+27 ml of a mixture, 0.21 M in mannitol, 0.07 M in sucrose and 0.1 mM in EDTA
⇩
Homogenized with a polytron homogenizer
⇩
Centrifuge 10 min, 700 g at 5°C
⇩
Supernatant was centrifuged 10 min, 8,000 g at 5°C
⇩
Sediments were briefly homogenized with 30 ml of the mixture
⇩
Centrifuged 10 min, 8,000 g at 5°C
⇩
Sediments were homogenized with appropriate volume of the mixture

acetaldehyde into the mitochondrial suspension. Both ethanol and acetaldehyde were redistilled before their uses. Blood was collected at the decapitation of rats for the determination of ethanol, acetaldehyde, or NEFA content. The alcohol content and the NEFA value of the serum were determined by the method of Widmark[10] and Dole[11], respectively. The acetaldehyde value was determined by the method of Stotz[12].

RESULTS

1. Body weight and levels of serum ethanol, acetaldehyde, and NEFA

The time course of increase in body weight of the ethanol fed group in comparison with that of the control group is shown in Fig. 1. The rats of the alcohol fed group became drunk and lost the appetite at the start of enforced ethanol administration, and continued being drunk for four or six hours, which possibly caused the decreased body weight compared to the control group; however, they regained their appetite a week later. Ethanol, acetaldehyde and NEFA in the serum were determined on the 24th day of the experiment after the animals were fasted for five hours. The blood ethanol content in the alcohol-fed group was 218 ± 3.7 mg/dl, the serum acetaldehyde level of the alcohol fed group was 0.7 ± 0.13 mg/dl, while those in control group were zero. Serum NEFA content of the alcohol fed group was markedly increased in comparison with that of control group (See Table 2).

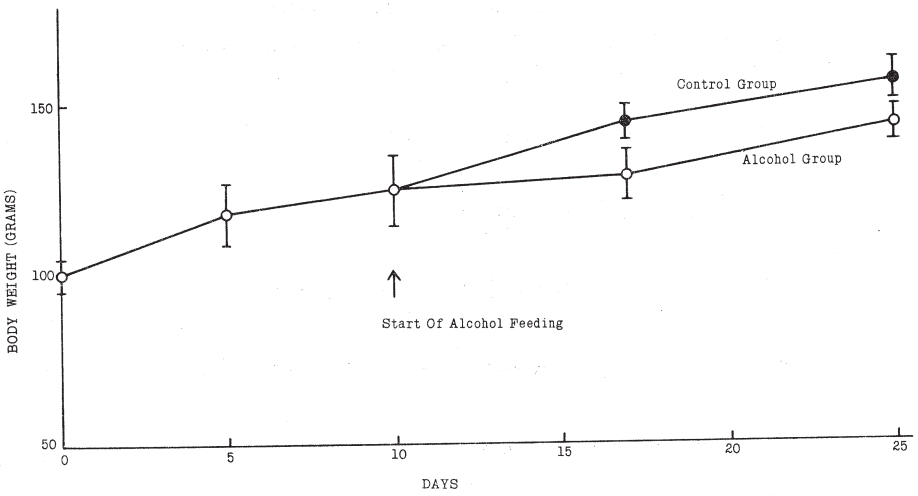

FIG. 1. Changes in body weights of rats in the alcohol fed group and the control group. Each point represents mean body weight±S.E. of six rats of both groups.

TABLE 2. Serum Content of Alcohol, Acetaldehyde, and NEFA

Group	Serum alcohol (mg/dl)	Serum acetaldehyde (mg/dl)	Serum NEFA (μEq/l)
The control group	0	0	790.7± 98.3
The alcohol group	218±3.7	0.7±0.13	1124.6±107.5

The values represent mean concentration±S.E. of six rats in both groups, and were measured 5 hours after injection of alcohol on 24th day.

TABLE 3. Respiratory Control Index and AMP/O Ratio of the Alcohol Fed Group and the Control Group

Addition		None		Bovine serum albumin	
Group	No.	R.C.I.	AMP/O	R.C.I.	AMP/O
The control group	1	4.9	1.02	5.1	1.16
	2	4.5	1.06	4.7	1.04
	3	6.4	0.98	6.5	1.08
	4	5.0	0.99		
	5	5.0	1.06		
	6	5.5	1.01		
	Mean ±S.E.	5.2±0.6	1.02±0.03	5.4±0.8	1.09±0.05
The alcohol fed group	1	2.4	0.88	2.4	0.89
	2	3.3	0.76	3.4	0.77
	3	3.2	0.80	3.0	0.80
	4	2.9	0.68		
	5	3.4	0.72		
	6	2.6	0.79		
	Mean ±S.E.	3.0±0.4	0.77±0.08	2.9±0.4	0.82±0.05

2. Respiratory control index and AMP/O ratio of the alcohol fed and the control group

The measured R.C.I. and AMP/O ratio obtained from the mitochondria of the alcohol-fed rats and those of the control rats are listed in Table 3. They were measured with and without bovine serum albumin in its final concentration, 0.5 mg/ml of mitochondrial suspension, however albumin did not affect both indexes in the alcohol-fed group and in the control group (Table 3). The data exclude a possibility that NEFA which is increased in the serum of ethanol fed rat exists in the mitochondrial sample causing the apparent uncoupling. If the possibility is true, the observed uncoupling should be recovered by the addition of albumin, as NEFA is readily combined with albumin.

3. The effect of ethanol addition in vitro on the mitochondrial suspension

The effect of ethanol addition *in vitro* on the normal rat liver mitochondria was tested. The final alcohol concentrations in these experiments were 20, 40, 80, 120, 160, and 200 μmoles/ml of medium. Alcohol addition more than 40 μmoles/ml in its final concentration caused a significant decrease of R.C.I. and

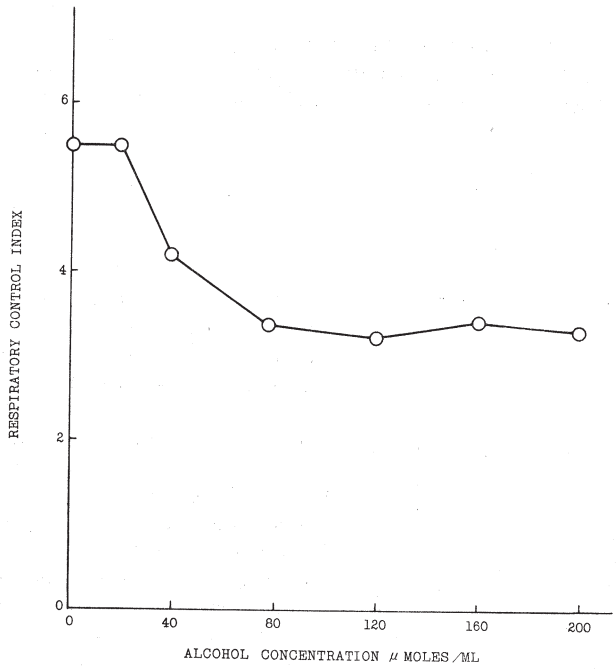

FIG. 2. The effect of alcohol addition *in vitro* on the mitochondrial respiratory control index. Each point represents the effect of addition of alcohol on the respiratory control index of the liver mitochondria obtained from the rats in the control group.

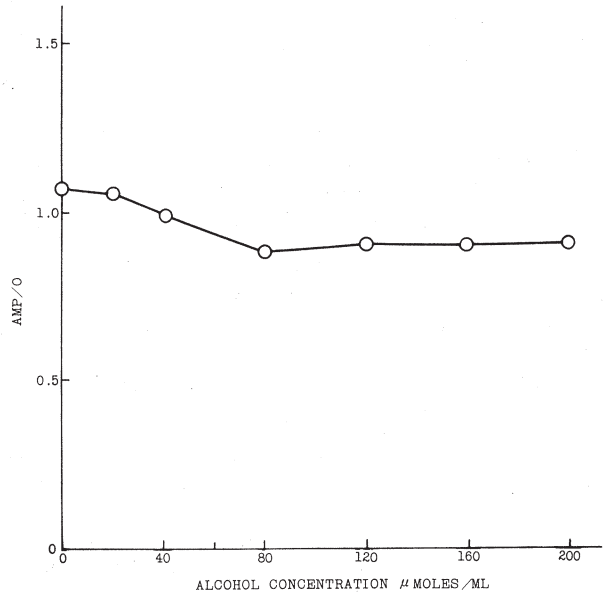

FIG. 3. The effect of alcohol on the mitochondrial AMP/O. Each point represents the effect of alcohol addition *in vitro* on the AMP/O of the liver mitochondria obtained from the control group.

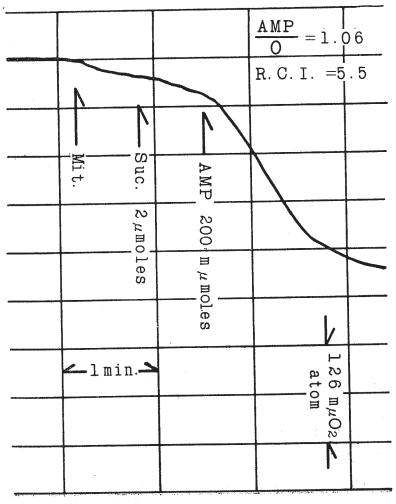

FIG. 4. Respiratory control and AMP/O of liver mitochondria of the rat in the control group. The reaction mixture was 0.3 M in mannitol, 10 mM in KCl, 10 mM in phoshate, 2 mM in $MgCl_2$, 0.25 mM in EDTA, it contained 2 mg protein of mitochondria per ml. The total volume was 5 ml, the temperature 20°C. The amounts of added substances per ml of the mixture are given on the figure. Changes in oxygen content in the mixture were recorded by a Beckman polarographic oxygen electrode (Clark type) with continuous stirring. The oxygen electrode was calibrated against air saturated distilled water at the appropriate temperature. pH of the whole reaction mixture was 7.4.

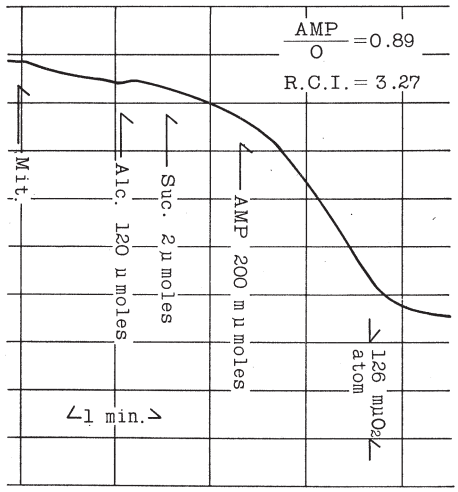

FIG. 5. Respiratory control and AMP/O of liver mitochondria, with the addition of alcohol (120 μmoles/ml of medium). The experimental conditions were those described in Fig. 4, except the addition of alcohol.

slight decrease of AMP/O ratio, as shown in Figs. 2 and 3.

The respiratory control of mitochondria without ethanol is shown in Fig. 4, while Fig. 5 is the chart with ethanol showing the impairment of oxidative phosphorylation.

4. The effect of addition of acetaldehyde on the mitochondrial suspension

R.C.I. and AMP/O ratio were calculated with and without various concen-

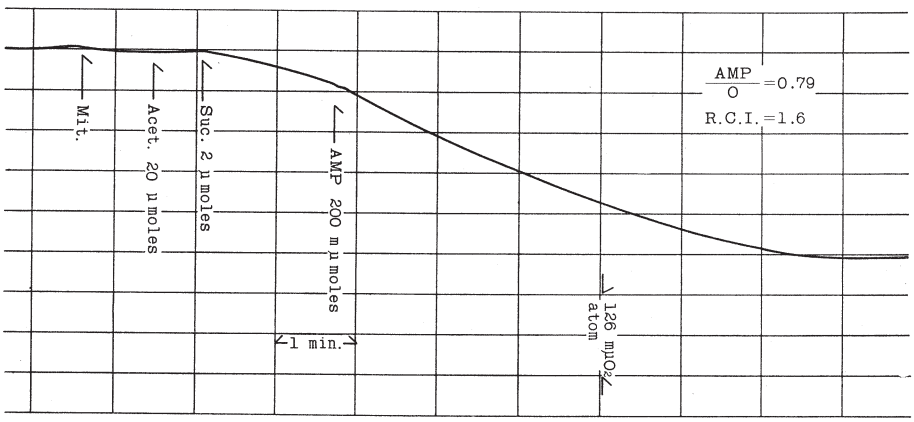

FIG. 6. Respiratory control and AMP/O of liver mitochondria of the rats in the control group in the presence of acetaldehyde. The experimental conditions were those described in Fig. 4, except the addition of acetaldehyde.

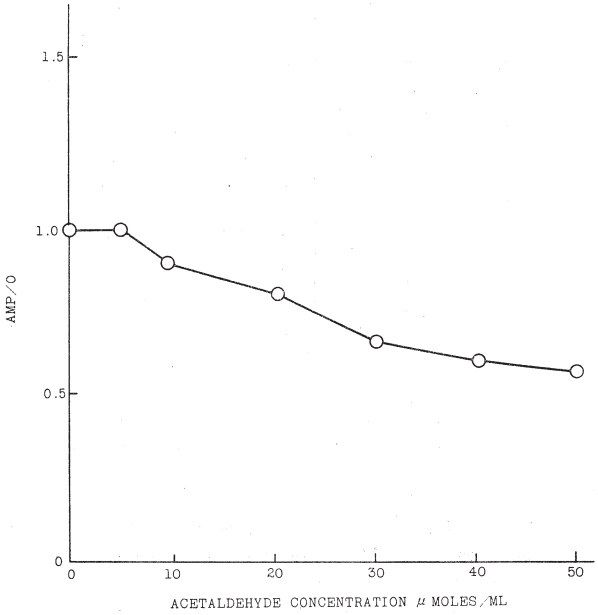

FIG. 7. The effect of acetaldehyde on the mitochondrial AMP/O. Each point represents the effect of addition of acetaldehyde on AMP/O of the liver mitochondria obtained from the rats in the control group.

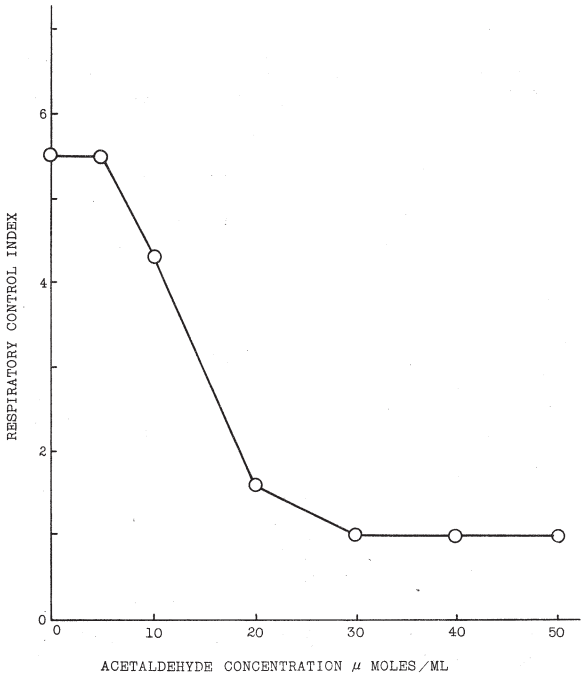

FIG. 8. The effect of acetaldehyde on the mitochondrial respiratory control index. Each point represents the effect of the addition of acetaldehyde on the respiratory control index of the liver mitochondria obtained from the rats in the control group.

trations of acetaldehyde.

As is shown in Figs. 7 and 8, the acetaldehyde of concentration more than 10 μmoles/ml of medium impaired both the R.C.I. and AMP/O ratio. Typical chart of impairment due to acetaldehyde is seen in Fig. 6.

DISCUSSION

The term, respiratory control, was started by Lardy and Wellman[13]. Chance and Williams[9] insisted that respiratory control index is a good indicator to show the intactness of mitochondria, although its precise mechanism has not been clarified. AMP/O is an indicator to represent the efficiency of the mitochondrial oxidative phosphorylation[14]. The decrease of these indexes could, therefore, be considered as some disorders in the mitochondrial oxidative phosphorylation mechanism. In the present communication, the authors found a significant decrease in both indexes in the liver mitochondria of alcohol fed rat compared to the normal one. Recently Kiessling[5] has observed swollen liver mitochondria by electron micrographic technique in his alcohol fed rats (90 days). The phosphorylation coupled to the oxidation of succinate in mitochondria from alcohol treated rats was the same with that in mitochondria from normal rats.

The discrepancy between Kiessling's results and authors might be due to the difference in alcohol doses and in feeding conditions. The decreased efficiency of oxidative phosphorylation in the alcohol fed group may be due to disorders in lipid metabolism reported by Brodie, *et al.*[2] As the long-chain unsaturated fatty acids are known as an uncoupling factor[15], the possibility of the uncoupling due to fatty acids was checked by adding bovine serum albumin to the reaction mixture. The results shown in Table 2 exclude this possibility, as the presence of albumin did not improve both indexes. The authors could not exclude the possibilities that fatty acid had induced irreversible changes on mitochondrial function enough to be unable to respond to the addition of albumin. It is remarkable that the *in vitro* addition of rather small amount of alcohol could decrease respiratory control. This finding agrees with Thore's reports[16] that exogenously added lower aliphatic alcohol had induced structural and functional changes of mitochondria. The authors' finding that acetaldehyde induced the uncoupling of mitochondria is very interesting. Judging from the effective concentration, it is scarecely possible that acetaldehyde has some roles to the mitochondrial function in alcohol fed rats *in vivo*. However, from the biochemical standpoint, the finding could be an possible tool to elucidate the mechanism of mitochondrial coupled respiration. The precise reports on the mechanism of the uncoupling due to acetaldehyde will be reported elsewhere. The OH-group of alcohol has dipolarity and due to its dipolarity, alcohol molecule is assumed to layer in the phase boundaries

between lipid and protein phases in the membrane of mitochondria. This is the region where the electron transport and energy transfer reactions takes place. The presence of alcohol may be assumed to change the high order conformational changes in mitochondria resulting in disorders of oxidative phosphorylation. The disturbed mitochondrial function of ethanol fed rats might be partly due to some metabolic disorder of liver, partly due to the toxic effect of alcohol on the mitochondria if the concentration of alcohol is high.

SUMMARY

1. Male albino rats fed with ethanol for two weeks in addition to ordinary diet showed the partially uncoupled oxidative phosphorylation in liver mitochondria.

2. Both ethanol and acetaldehyde added *in vitro* impaired respiratory control index and AMP/O of isolated liver mitochondria.

3. Judging from the minimum effective concentration for the uncoupling, not acetaldehyde, but ethanol must have some physiological significance for the disturbance of mitochondrial function.

These results were discussed in connection with alcoholic liver damage.

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