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Kozo Inaba*

*Okayama University,

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Abstract

Respiration, activity of oleate oxidation and composition of the total fatty acids of rat liver were investigated in 3'-Me-DAB feeding. 1. Oxidative phosphorylation of rat liver mitochondria decreased temporarily at relatively earlier stages (about 2 to 3 weeks) in 3'-Me-DAB feeding. 2. The activity of oleate oxidation of rat liver mitochondria decreased rapidly to about one third of that in control groups after the start of 3'-Me-DAB feeding. 3. In the composition of the total fatty acids of rat liver, the proportion of oleic acid increased in 3'-Me-DAB groups. 4. Unknown octadecamonoenoic acid was observed in liver mitochondria of rat fed on 3'-Me-DAB. 5. Proportions of oleic and palmitoleic acids in liver tumors and mitochondria of liver tumors induced by 3'-Me-DAB feeding increased remarkably in contrast with decrease in those of palmitic and eicosapolyenoic acids. 6. A possibility was discussed about how higher level of oleate in the liver cells in azo dye feeding may be concerned with the tumor induction.

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CHANGES OF FATTY ACID METABOLISM AND OXIDATIVE PHOSPHORYLATION OF RAT LIVER MITOCHONDRIA DURING 3′-Me-DAB FEEDING

Kozo INABA

Department of Biochemistry, Cancer Institute of Okayama University Medical School, Okayama, Japan
(Director: Prof. M. Yamamoto)

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Many works have been made in regard to metabolic changes during 3′-Me-DAB feeding. In carcinogenesis by azo dyes, activities of various enzymes were assayed, demonstrating significant changes in some and no significant changes in others[1]. DESSI reported[2] a slight, gradual decrease or no significant change in respiration and an increase in glycolysis during azo dye feeding. There are few reports, however, on the relationship between the metabolic change and the mechanism of the induction of tumor in rat liver with azo dye feeding.

Recently, ARCOS *et al.*[3] reported that feeding of 3′-Me-DAB to rats resulted in the alteration of the liver mitochondrial structure, namely, the swelling ability decreased temporarily and reached minimum level at a relatively earlier stage (about 4 weeks), and they found that the minimum period of 3′-Me-DAB feeding for tumor induction was in accord with the time of minimum mitochondrial swelling. Low values for the thyroxine-induced swelling were also observed in the mitochondria from hepatoma induced by 3′-Me-DAB and from various neoplastic livers[4].

In the present paper the changes of oxidative phosphorylation and fatty acid metabolism of rat liver during 3′-Me-DAB feeding are described.

MATERIALS AND METHODS

Feeding with 3′-Me-DAB : Each rat, weighing 180 to 220 g was usually fed on polished rice for a month before the experiment and fed on the rice or the latter supplemented with 3′-Me-DAB (0.06%) for 46 days under constant conditions. In some experiments, rats were fed on the semi-synthetic diet containing 20 g casein, 60 g starch, 60 g sucrose, 10 g agar, 2 g NaCl, 10 g shortening butter, 60 mg 3′-Me-DAB and 100 ml water. From the time rats were fed on the diet, the experiments were carried out periodically, usually at intervals of a week.

Endogenous respiration of rat liver homogenate: Livers were removed from 3′-Me-DAB fed rats (10 animals) at various stages and were homogenized with an equal volume of 0.25 M sucrose. Oxygen uptake was measured with Warburg apparatus and the reaction mixture was composed as follows; 2.0 ml of homogenate and 0.5 ml of Krebs-Ringer phosphate buffer (pH 7.4) in the main chamber and 0.2 ml of 20% NaOH solution in the center well. Incubation was carried out with air at 25°C for 30 minutes.

Oxidative phosphorylation and oxidation of sodium oleate by liver mitochondria: Liver mitochondria were separated from 3′-Me-DAB fed rats at various stages with the method of HOGEBOOM[5]. Mitochondria derived from five livers (about 25 g wet wt.) were suspended in 25 ml of 0.25 M sucrose.

Oxidative phosphorylation of the mitochondria was measured with a polarographic method[6]. The mitochondria suspension (0.1 ml) was added to 2 ml medium containing 0.1 M of sucrose, 20 mM KCl, 1 mM $MgCl_2$, 40 μM EDTA and 5 mM Tris-HCl buffer (pH 7.4). Other additions to the reaction mixture are shown in the legends to the figures and tables. Incubation was carried out at 25°C.

Total nitrogen of mitochondria was determined with semi-micro Kjeldhal method[7].

Fatty acid composition of liver and liver mitochondria: Total lipids were extracted from the rats at the same stages as the foregoing experiment with the method of FOLCH *et al*[8]. Fatty acid composition of total lipid was analyzed by gas-liquid chromatography after saponification for 3 hours under a nitrogen gas stream and esterification with diazomethane.

RESULTS

Oxidative phosphorylation of rat liver: Table 1 shows the changes of respiration of rat liver during 3′-Me-DAB feeding. Both of endogenous respiration of liver homogenate and oxidative phosphorylation of liver mitochondria in the absence or presence of 10 mM of sodium succinate decreased temporarily to the minimum levels at relatively earlier stages during 3′-Me-DAB feeding.

Oxidation of sodium oleate by rat liver mitochondria: As it has been clarified that the oxidative phosphorylation of rat liver mitochondria decreased temporarily at a relatively earlier stage of 3′-Me-DAB feeding, with regard to fatty acid metabolism which plays an important role in the endogenous energy source and the structure of mitochondria, the following experiments have been carried out with polarographic method and gas-liquid chromatography.

As shown in curve B of Fig. 1, sodium oleate (100 mμ moles) was oxidized by mitochondria and this oxidation was remarkably inhibited by the addition of

Table 1 Respiration of rat liver homogenate and mitochondria during 3′-Me-DAB feeding

Rats were fed on the semi-synthetic diet containing 0.06% 3′-Me-DAB in experiment 1 or on polished rice supplemented with 0.06% 3′-Me-DAB in experiment 2. Oxidative phosphorylation of rat liver mitochondria was determined by polarographic method. $Q_{O_2}(N)$ of rat liver homogenate in experiment 1 was determined with manometric method. Details were described in the text.

Exptl. No.	Days of 3′-Me-DAB feeding		$Q_{O_2}(N)$	P/O
1	control		49.6	2.3
	3′-Me-DAB	7	47.6	2.2
		17	35.4	1.0
		23	14.2	1.1
		28	45.2	2.5
		41	56.2	—
2	control		—	2.4
	3′-Me-DAB	9	—	2.3
		16	—	1.7
		23	—	1.7
		29	—	2.4
		36	—	2.4

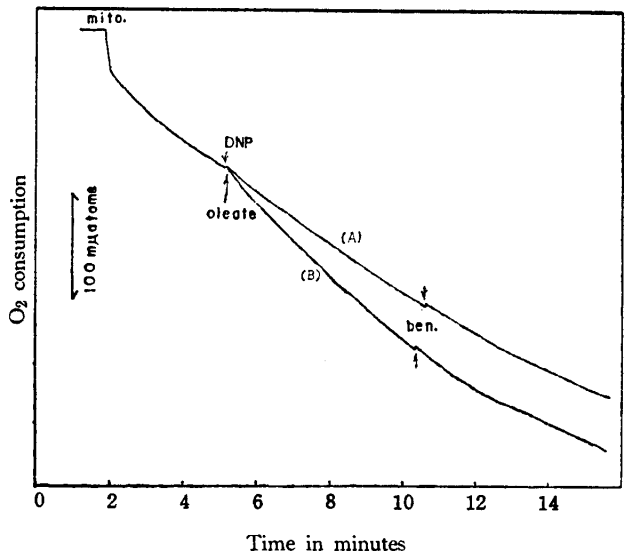

Fig. 1 Effect of sodium benzoate on oleate oxidation. Tracings of platinum electrode. Mitochondria (4 mg protein) were added to 2 ml of basic medium containing 5 mM Pi. Additions made as indicated by the arrows. Abbreviations: mito, mitochondria; DNP, 30 mμ moles dinitrophenol; oleate, 100 mμ moles sodium oleate; ben, 20 μ moles sodium benzoate. (A): Release of the endogenous respiration induced by DNP. (B): Oleate oxidation by mitochondria.

sodium benzoate (10^{-2} M). Release of the endogenous respiration of mitochondria induced by DNP (1.5×10^{-5} M) was scarcely inhibited by the addition of sodium benzoate (curve A in Fig. 1).

Activity of oleate oxidation in 3′-Me-DAB feeding groups, which was indicated by oxygen consumption per minute per mg protein of mitochondria in the optimum concentration of oleate, decreased to about one third that in control groups (Table 2).

Table 2 Activity of oleate oxidation by rat liver mitochondria during 3′-Me-DAB feeding

Rats were fed on polished rice supplemented with 0.06% 3′-Me-DAB. Oxygen uptake of mitochondria by the addition of sodium oleate at an optimum concentration was determined by a polarographic method. Details are described in Fig. 1 and the text.

Group	Days of 3′-Me-DAB feeding	Optimum concentration of oleate	O_2 consumption
		μM	mμ atoms/min. /mg prot.
Control 1	—	100	18.4
2	—	150	24.4
3′-Me-DAB	9	20	5.0
	16	30	8.9
	23	50	8.3
	29	70	6.9
	36	70	6.6

Composition of the total fatty acids : The total fatty acids of rat liver and liver mitochondria were analyzed in connection with changes in activity of the oleate oxidation during the feeding of 3′-Me-DAB.

As shown in Table 3, main long chain fatty acids in the total lipid fraction of rat liver were composed of palmitic, palmitoleic, stearic, oleic, linoleic and eicosapolyenoic acids. The proportion of oleic acid in 3′-Me-DAB groups increased in contrast with that of control groups.

Table 4 shows fatty acid compositions of the total lipid fractions of the liver mitochondria of rats in 3′-Me-DAB feeding. Main long chain fatty acids were consisted of palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and esicosapolyenoic acids. No significant changes in oleic acids were observed by 3′-Me-DAB feeding, however, unknown octadecamonoenoic acid appeared in the mitochondria of 3′-Me-DAB feeding groups (Figs. 2 a and b).

Table 5 shows an analysis of total fatty acids of liver tumors induced by 3′-Me-DAB feeding and of the mitochondria. Main long chain fatty acids of these fractions were composed of palmitic, palmitoleic, stearic, oleic, linoleic and eicosapolyenoic acids. Proportions of oleate and palmitoleate increased

Table 3 Fatty acid composition of rat liver during 3′-Me-DAB feeding

Rats were fed on the semi-synthetic diet for 7 days and then were fed on the diet supplemented with 0.06% 3′-Me-DAB in experiment 1, and in experiments 2 and 3, rats were fed on polished rice for 30 and 7 days, respectively, and then were fed on polished rice supplemented with 0.06% 3′-Me-DAB. Total lipids of rat liver were extracted by the method of Folch *et al.*[8]. Fatty acids were analyzed by gas-liquid chromatography. Details are described in the text.

Exptl. No.	Group	Days of 3′-Me-DAB feeding	Percentage composition					
			$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:poly.}$
1	Control 1	—	14.8	8.9	12.6	18.3	24.6	19.6
	2	—	19.7	4.0	17.0	22.2	14.5	18.8
	3	—	22.2	5.4	15.5	19.9	15.0	20.3
	3′-Me-DAB	7	15.7	7.0	14.7	20.5	25.0	15.9
		17	18.3	3.1	17.8	26.4	15.1	15.6
		23	19.1	3.7	17.1	25.2	13.3	11.5
		28	18.8	7.7	17.7	26.9	13.4	10.2
		41	24.1	5.0	17.4	28.5	11.5	10.8
2	Control 1	—	33.1	5.2	16.4	26.6	9.1	9.6
	2	—	31.9	7.1	19.0	20.5	9.4	12.3
	3′-Me-DAB	9	28.8	5.2	14.9	33.2	9.8	8.2
		16	30.9	4.6	9.8	34.2	10.4	10.1
		23	31.0	3.9	21.2	27.0	9.1	7.7
		29	37.2	7.7	18.6	23.6	8.0	4.6
		36	35.0	10.0	15.0	22.1	10.0	7.9
3	Control 1	—	37.6	3.0	34.1	15.2	5.0	—
	2	—	34.7	5.0	29.4	23.6	5.0	—
	3′-Me-DAB	6	34.4	2.9	28.1	23.7	10.4	—
		20	36.7	2.8	28.1	22.0	10.6	—
		30	28.1	5.2	15.9	37.7	5.3	—
		39	30.7	4.7	18.3	37.5	3.4	—

Table 4 Fatty acid composition of rat liver mitochondria during 3′-Me-DAB feeding

Rats were fed on polished rice for 30 days and then were fed on polished rice supplemented with 0.06% 3′-Me-DAB. Rat liver mitochondria were isolated, and the total lipids were extracted by the method of Folch *et al.*[8]. Details are described in the text.

Group	Days of 3′-Me-DAB feeding	Percentage composition								
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:1'}$	$C_{18:2}$	$C_{18:2'}$	$C_{20:poly.}$ 1	$C_{20:poly.}$ 2
Control 1	—	27.4	8.3	9.5	11.8	trace	24.4	1.8	16.3	—
2	—	43.8	10.7	22.4	17.4	0	4.4	0	1.5	—
3′-Me-DAB	16	23.4	7.4	11.2	12.2	5.4	20.0	4.4	11.6	4.3
	23	46.4	10.0	11.1	12.0	6.4	8.6	—	5.8	—
	29	47.2	13.0	12.8	10.2	4.6	7.9	0.8	4.2	0.7
	36	19.3	11.2	9.2	20.8	6.4	20.0	5.1	4.7	3.3

remarkably in contrast with decrement of those of palmitic and eicosapolyenoic acids in the both fractions of 3′-Me-DAB feeding groups.

Table 5 Fatty acid composition of the liver tumors induced by 3′-Me-DAB feeding and of the mitochondria.

Rats were fed on the semi-synthetic diet for 7 days and on the diet supplemented with 0.06% 3′-Me-DAB for 41 days, thereafter they were fed on commercial semi-synthetic diet for 272 days. Tumor parts were separated from non-tumor parts of liver.

	Percentage composition								
	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:2'}$	$C_{18:3'}$	C_{20}:poly
Whole homogenate									
non-tumor parts	25.4	2.5	1.0	18.9	14.4	20.1	1.6	0.9	13.7
tumor parts	24.0	4.4	0.7	13.2	21.1	23.4	0.8	0.9	10.4
Mitochondria									
non-tumor parts	32.7	4.0	1.6	15.6	9.7	25.2	2.2	2.2	5.7
tumor parts	27.6	5.6	2.7	15.7	12.9	22.5	3.4	2.8	5.4

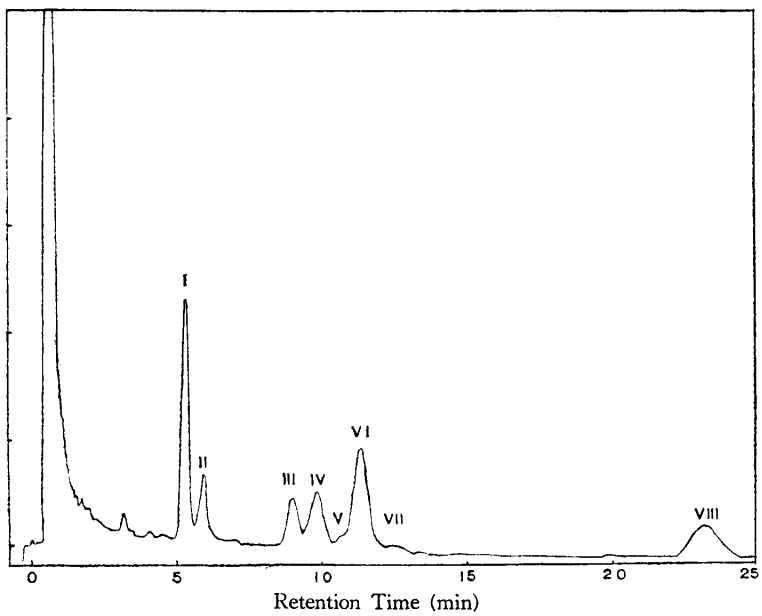

Fig. 2 (a) Typical gas-liquid chromatogram of the fatty acids in the liver mitochondria of the control rat. Rats were fed on polished rice for 45 days, Peak I, palmitic acid; Peak II, palmitoleic acid; Peak III, stearic acid, Peak IV, oleic acid; Peak V, octadecamonoenoic acid (unknown); Peak VI, linoleic acid; Peak VII, octadecadienoic acid (unknown); Peak VIII, eicosapolyenoic acid 1 (unknown).

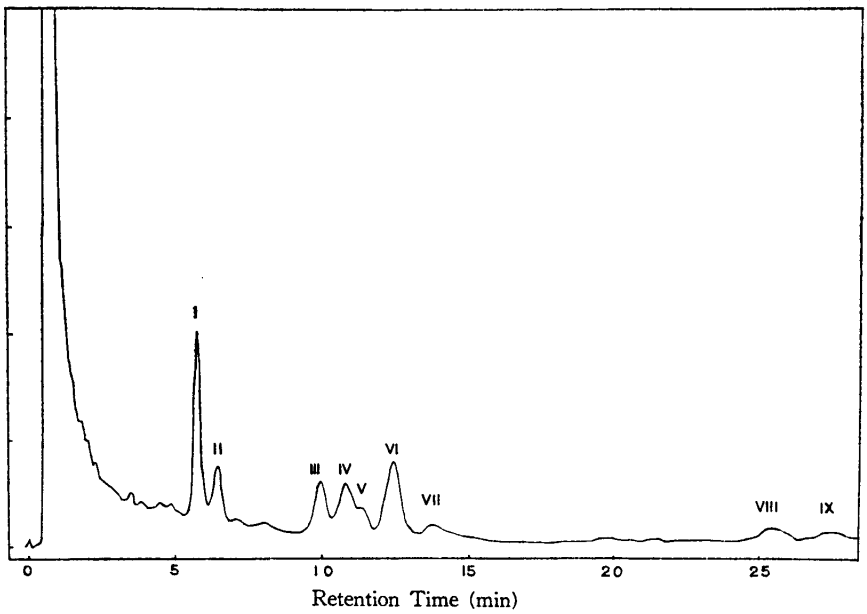

Fig. 2 (b) Typical gas-liquid chromatogram of the fatty acids in the liver mitochondria of the 3′-Me-DAB fed rat. Rats were fed on polished rice for 30 days and then were fed on polished rice supplemented with 0.06% 3′-Me-DAB for 16 days. Peak I, palmitic acid; Peak II, palmitoleic acid; Peak III, stearic acid; Peak IV, oleic acid; Peak V, octadecamonoenoic acid (unknown); Peak VI, linoleic acid; Peak VII, octadeca dienoic acid (unknown), Peak VIII, eicosapolyenoic acid 1 (unknown); Peak IX, eicosapolyenoic acid 2 (unknown).

DISCUSSION

It has been considered from many reports that no significant changes take place in the respiration of rat liver during carcinogenesis. However, there are few data on the changes in the oxidative phosphorylation, especially, at relative earlier stages of 3′-Me-DAB feeding. As described in this paper, a temporary decline of oxidative phosphorylation of rat liver has taken place at a relatively earlier stage of the 3′-Me-DAB feeding, and this phenomenon would be related to that of the swelling ability of mitochondria to various swelling agents[3].

There are few reports with regard to the changes of fatty acids, which play an important role in the energy source and the structural integrity of mitochondria, in azo dye feeding. MEDES *et al.*[9] reported that no significant changes in fatty acid contents and the incorporation of acetate-2-C^{14} into fatty acids had occurred throughout the mass of liver cells prior to the appearance of tumor cells in the DAB feeding. As shown in this paper, however, a decline was observed in the oleate oxidation of liver mitochondria of rat fed on 3′-Me-DAB.

In fact, the proportion of oleate in the fatty acid composition of the rat liver fed on 3′-Me-DAB is higher than that of control groups. Furthermore, it is noteworthy that the proportion of oleate in the liver tumors induced in 3′-Me-DAB feeding is higher than that of the non-tumor parts of the liver. This fact can be supported by the recent report of VEERKAMP *et al.* [20] concerned with the increment of the proportion of oleate to the total fatty acids in various tumor cells. Therefore, it can be assumed that the increment of oleate in the DAB feeding might be associated with the carcinogenesis and the derangement of the energy metabolism. Furthermore, it is possible to consider that many factors, including protein and lipid, would be concerned with the structural integrity of mitochondria. The higher contents of cystine[11] and the higher proportion of oleate in mitochondria of liver tumor induced by azo dye feeding would be associated at least with the rigidity of the mitochondria.

Further investigations are necessary to elucidate the mechanism of changes in the function of rat liver mitochondria during carcinogenesis.

SUMMARY

Respiration, activity of oleate oxidation and composition of the total fatty acids of rat liver were investigated in 3′-Me-DAB feeding.

1. Oxidative phosphorylation of rat liver mitochondria decreased temporarily at relatively earlier stages (about 2 to 3 weeks) in 3′-Me-DAB feeding.
2. The activity of oleate oxidation of rat liver mitochondria decreased rapidly to about one third of that in control groups after the start of 3′-Me-DAB feeding.
3. In the composition of the total fatty acids of rat liver, the proportion of oleic acid increased in 3′-Me-DAB groups.
4. Unknown octadecamonoenoic acid was observed in liver mitochondria of rat fed on 3′-Me-DAB.
5. Proportions of oleic and palmitoleic acids in liver tumors and mitochondria of liver tumors induced by 3′-Me-DAB feeding increased remarkably in contrast with decrease in those of palmitic and eicosapolyenoic acids.
6. A possibility was discussed about how higher level of oleate in the liver cells in azo dye feeding may be concerned with the tumor induction.

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