RESPIRATION OF CYCLOPROPENOID FATTY ACIDS *IN VITRO*

by

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ABSTRACT

Cyclopropenoid fatty acids (CPFA) have been shown to induce a variety of physiological disorders when fed to animals. Among these are reduced growth, abnormalities in the reproductive process and alterations of lipid metabolism.

This study attempted to measure the oxygen consumptions of the liver mitochondria with the substrates of cyclopropenoid fatty acid and oleic acid during beta-oxidation. The difference in oxygen consumption may give a crude idea about the difference in metabolic rate between cyclopropenoid and mono-unsaturated fatty acid.

In this study, female albino rats of Sprague-Dawley strain were divided into two groups, one group was fed four per cent safflower oil as the sole source of fat in the diet, the other group was fed one per cent safflower oil and three per cent Sterculia foetida oil.

Fresh rat liver mitochondria preparations were used as the enzyme source. The 4×10^{-4} M concentrations of oleic acid or methyl sterculate were as substrates.

In the media containing liver mitochondria from the safflower oil fed rats, oxygen consumption was two to two and one half times higher with substrate of methyl sterculate than with oleic acid. The same results was observed when the liver mitochondria of CPFA fed rats were used in the incubation media.

The incubation media analyzed at 0, 20, 40, and 80 minutes showed a ratio of stearic acid to oleic acid which was higher for the CPFA fed rats than in those fed safflower oil using the same substrate; also, this ratio was found to be higher when methyl sterculate was used as the substrate than when oleic acid was used.

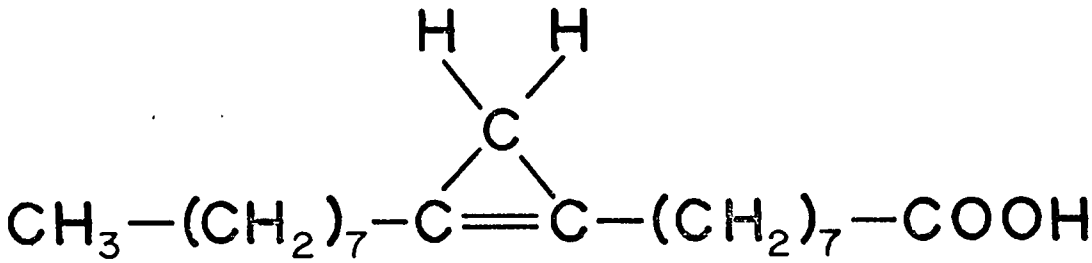
INTRODUCTION

Cyclopropene compounds have been demonstrated to have the following biological effects: pink-white discoloration and associated defects in eggs (1,2), depression of egg production (3), depression of growth and delay of sexual maturity in female hens (3), and rats (4), decreased chicken hatchability and mammalian fertility (5), altered fat metabolism and change of lipid makeup in the animal body.

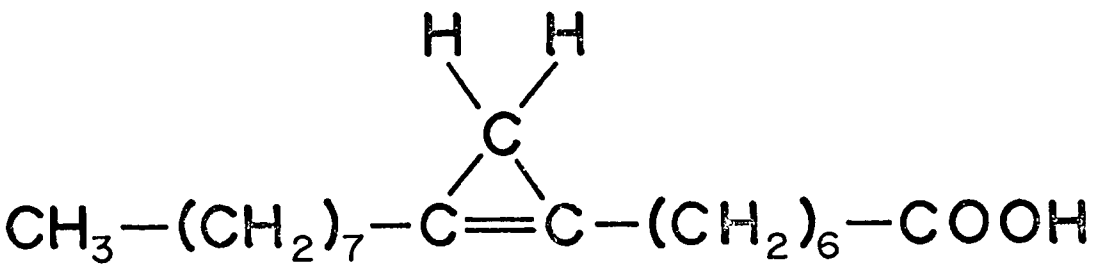
The naturally occurring cyclopropenoid fatty acids (CPFA) known to be associated with the reported disorders are sterculic and malvalic acids (Fig. 1). The concentration of sterculic acid in the oil extracted from Sterculia foetida seeds is approximately thirty to fifty per cent depending on the method of analysis. Malvalic acid is also present in Sterculia foetida seeds oil but at much less concentration; it is about one-tenth the concentration of the sterculic acid.

A color test introduced by Halphen (1897) was originally used for the detection of cottonseed oil in mixtures of vegetable oils (6). Later, Brooke, Smith (1957), Shenstone, Vickery (1959), and Nordby (1963) have indicated that the cyclopropene ring was essential for a positive Halphen reaction (7,8,9). The Halphen test has been used as a method to detect the presence of cyclopropene fatty acids.

It has been reported that more than forty-eight species of plants contain cyclopropene fatty acids in their seed oils (1). The



STERCULIC ACID



MALVALIC ACID

Fig. 1. Cyclopropenoid Fatty Acids

isolation of cyclopropene fatty acids from microorganisms has not been reported; however, there are some indications that cyclopropene fatty acids may be the intermediate in the formation of a cyclopropanoid fatty acid which has been isolated from several genera of bacteria (10,11). Cyclopropene fatty acids have been detected in animals which have been fed diets containing cyclopropene fatty acids (12).

Harrington and Adrience (13) reported in 1897 that a hard lard was obtained from pigs which had been fed on a cotton-seed meal diet. Similar effects in cows, hens and fish were reported (14,15,16). The fatty acids in the experimental animals contained more stearic acid and linoleic acid and less oleic acid than the control animals. This effect on fat metabolism was further studied by Evans et al. (15), and the evidence supported the view that the cyclopropenoid compounds were responsible for the fatty acid changes. The cyclopropene ring is the essential requirement for inhibition of the fatty acid desaturation (17).

In 1964, Kircher suggested an explanation for the biological activity of the cyclopropene compounds. He used methyl sterculate and mercaptans to demonstrate that a reaction between the thiol group and the cyclopropene double bond can occur and postulated that reactions may occur with available sulfhydryl groups of the physiologically active proteins (18).

In 1967, Allen and Johnson (19) have shown that the stearic acid desaturase system was specifically inhibited by cyclopropene fatty acid at low concentrations and the inhibition was reported to be

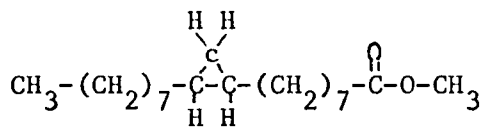
irreversible. Raju and Reiser postulated in 1967 (20) that the active principle in Sterculia foetida oil irreversibly binds the thiol group of acyl desaturase enzyme. The thiol group of the multienzyme complex of fatty acid synthetase may be less reactive than the thiol group of acyl desaturase.

In 1969, Johnson et al. (17) reported that the hen liver preparations which desaturated stearic acid at the 9,10 position to form oleic acid also could desaturate other saturated fatty acids of carbon chain length from 12 to 20 and 22 at the same 9,10 position. This suggested that the site of desaturation was independent of the number of methylene groups beyond the 9,10 position. Similar results were obtained by Howling, Morris, and James (21). Thus they concluded that a direct attachment of the saturated fatty acid or of the CoA ester, or of the acyl carrier protein thiol ester occurred with the desaturase enzyme complex. They also found that the maximum desaturation occurred with the C_{14} fatty acid substrate as well as the C_{17} and C_{18} fatty acid. This suggested the presence of at least two desaturating systems in the preparation of hen liver. The cyclopropene fatty acids inhibited the desaturation of all the fatty acids at the 9,10 position, but desaturation at the 10,11 and 11,12 position was affected only slightly.

The effects of cyclopropene fatty acids on the cause and development of cancer has been investigated by Sinnhuber et al. (22). In 1968 they reported that the cyclopropene fatty acids fed 220 parts per million in an aflatoxin-containing diet greatly increased the incidence and growth of hepatoma in rainbow trout.

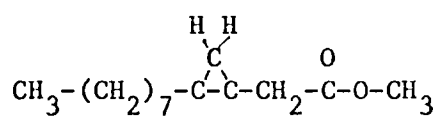
Labeling of cyclopropenoid fatty acids with radioactive C^{14} in the methylene group could provide the biochemical tool to study the mechanism of biological activity of cyclopropenoid fatty acids. Biological labeling of the cyclopropenoid acids (23,24) gave disappointing yields. Further attempts are being made to chemically snythesize the labeled cyclopropenoid fatty acids (18,25).

The chemical structure of cyclopropenoid fatty acids is quite similar to cyclopropanoid fatty acids. In 1965, Wood and Reiser (26) fed female rats a specific diet which contained the synthesized and radioactive C^{14} labeled racemic methyl cis-9, 10-methylene octadecanoate (CMO) and racemic methyl trans-9, 10-octadecanoate (TMO). The biodegradation products they found were racemic methyl cis and trans-3, 4-methylene dodecanoate (CMD and TMD) respectively. The metabolis products are apparently the result of the inabilities of the beta-oxidation enzyme system to continue past the cyclopropane ring in the fatty acid chain.



CMO or TMO

β-oxidation



CMD or TMD

Now the question is, "Does cyclopropenoid fatty acid have the same metabolic fate as cyclopropanoid fatty acid in the animal body?" The measurements of oxygen consumption of cyclopropenoid fatty acid during beta-oxidation should give indication if the oxidation proceeds past the cyclopropene ring.

The mammal burns fatty acids to CO_2 and H_2O at the expense of molecular oxygen; the stoichiometry of the conversion of oleic acid to CO_2 and H_2O is given by the following equation:

$$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-COOH + 25\ 1/2\ O_2$$

$$\downarrow$$

$$18\ CO_2 + 17\ H_2O$$

The catabolism of fatty acids to CO_2 and H_2O occurs by means of the sequential combination of two multienzyme systems. The beta-oxidation cycle converts the fatty acid containing an even number of carbon atoms totally to acetyl groups in thioester linkage with CoA (Coenzyme A). Then, the tricarboxylic acid cycle converts the acetyl moiety of acetyl-CoA to CO_2 and H_2O (27). The process of fatty acid oxidation is known to be localized in mitochondria. The general scheme of the beta-oxidation cycle is summarized in Fig. 2.

The amount of oxygen consumed as well as the amount of CO_2 produced in the catabolism of a fatty acid is proportional to its chain length. Because of the similarities of the position of the double bond

and the number of carbons, oleic acid and methyl sterculate should require a similar amount of oxygen during their metabolic processes if they both are catabolized in the same manner.

These experiments were designed to find out if there is a difference between the oxidation of cyclopropenoid fatty acid and mono-unsaturated-straight chain fatty acid in animal body.

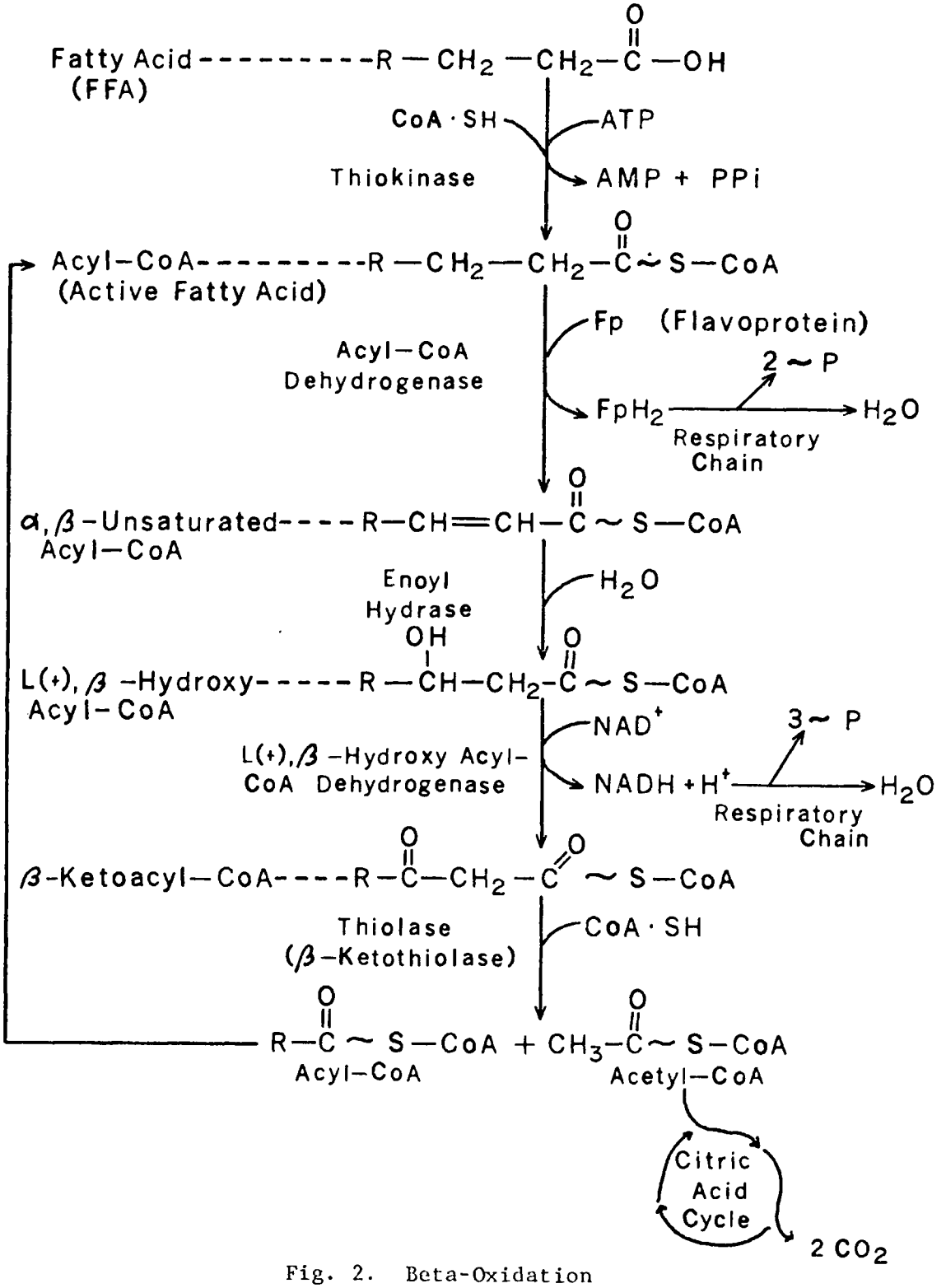


Fig. 2. Beta-Oxidation

METHODS, MATERIALS AND EXPERIMENTAL DESIGN

Animals

Female albino rats of Sprague-Dawley strain were used in these experiments. The rats were maintained on a standard laboratory diet until they were one month old, then they were divided into two groups (control and experimental) of comparable weight and were fed the control and experimental diets (Table 1). The rats were placed in sterilized, individual hanging cages, two rats per cage. The room temperature was maintained at 27°C by means of centralized heating and cooling. The *in vitro* studies were started after the rats were on the assigned diet for a minimum of two months. Both groups were maintained on a four per cent fat diet. Whereas the source of fat in the control group was four per cent safflower oil only (safflower oil diet), the experimental group was one per cent safflower oil and three per cent *Sterculia foetida* oil (CPFA diet).

Composition of the Diets

The composition of the diets is given in Table 1 and Table 2 (Schneider 1962, Sheehan and Vavich 1965, Sheehan 1967). The diets contained four per cent added fat. The vitamin mix and salt mix were prepared separately and then mixed with cerelose. The diets were mixed in a Hobart dough-type mixer. The diets with all ingredients except the oil were passed through a hammer mill to assure homogeneously

Table 1. Composition of Diets

Components	Control Diet	Experimental Diet
Casein[a]	22.00	22.00
Fat: Safflower oil	4.00	1.00
Sterculia foetida oil		3.00
Glucose-monohydrate[b]	61.00	61.00
Non-nutritive fiber[c]	4.00	4.00
Salt-mixture[d]	4.00	4.00
Vitamin mixture[e]	5.00	5.00
Total	100.00	100.00

[a]Vitamin free; Nutritional Biochemicals Corp., Cleveland, Ohio.

[b]Cerelose 2001; Corn Products Company, New York.

[c]Solka-Floc; Brown Company, Boston, Massachusetts.

[d]Jones and Foster (1942), with NaF added to give 10 ppm in salt mixture (in 4).

[e]Vitamin mixture, supplies per 100 g of diet (see Table 3).

Table 2. Vitamin Mixture Ingredients

Ingredients	mg/100g Diet	Ingredients	mg/100g Diet
Thiamine hydrochloride	0.40	Folic acid	0.20
Riboflavin	0.50	Brotin	0.02
Niacinamide	5.00	Vitamin B_{12}	10.00
Pyridoxine hydrochloride	0.25	0.1% trit in Mannitol	
Calcium pantothenate	2.00	Menadione	0.20
Choline bitartrate	200.00	α-tocapherol	5.00
Inositol	100.00	Vitamin A[a]	1000 IU
P-amino benzoic acid	10.00	Vitamin D_2[b]	120 IU

[a]PGB-10; Distillation Products Industries, Rochester, N.Y.

[b]Super Nopdex-30; NOPCO Chemical Company, Richmond, California.

powdered diets. All diets were stored at 4°C in plastic bags. Food was fed fresh daily. The diets of the experimental and the control animals were all isocaloric. Determinations of moisture, caloric density, fat, and nitrogen on the mixed diets were carried according to AOAC and the results are shown in Table 3.

Table 3. Analysis of Diet

Diet	Avg. % Moisture	Avg. Caloric Content Per Gram	Avg. % Protein	Avg. % Oil
4% safflower oil diet	7.18	4.13	18.4	3.96
1% safflower oil +3% *Sterculia foetida* oil diet	7.43	4.07	19.3	3.86

Preparation of the Sterculia Foetida Oil

The oil was extracted from *Sterculia foetida* seeds obtained from the Philippine Islands.[1] The whole seeds were ground and extracted with three liters of Skellysolve F per kilogram of seed. The oil-rich extracts were removed, and the residue filtered on a medium-porosity, sintered-glass filter and washed several times with solvent. After three successive, overnight extractions the extracts were combined and the solvent removed in a rotary film flash evaporator under reduced pressure. The final traces of solvent were removed with a high vacuum oil pump in a water bath at 35°C. The oils were stored at 4°C under

1. Zoilo C. Fraga, Forestry College, Laguna, Philippine Islands.

nitrogen. Cyclopropene fatty acids determined by the modified Halphen test (4) ranged from thirty-three to thirty-five per cent.

Preparation of Methyl Sterculate

The method was essentially the same as described by Kircher (18). Sterculia foetide oil was trans-esterified in dry methanol with sodium methoxide for three days at room temperature. Then glacial acetic acid (0.082 ml to 1 gm of Sterculia foetida oil) was added, the solution was diluted with methanol (18 ml to 1 gm of Sterculia foetida oil) and urea (1.2 gms of urea to 1 gm of Sterculia foetida oil) was added and dissolved at room temperature. The solution was cooled to -16°C in a freezer. The liquid was then filtered from the solid and cooled successively to -30°C and -45°C in a dry ice-acetone bath. Precipitates from the latter two low temperature crystallizations were combined, washed with water to remove urea and redissolved in the methanol. This solution was cooled to -45°C to precipitate pure methyl sterculate.[2]

Homogenates and Mitochondria

These were prepared according to Hogeboom (28). Rats were killed by cervical fracture. The livers removed quickly and chilled by immersion in 0.25 M sucrose at 0°C and were then blotted, minced with scissors into pieces about 0.5 cm in thickness and weighed. Cold 0.25 M sucrose (9 ml) were added to each 1.0 gm of liver and the tissue

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2. Methyl sterculate was made according to Kircher's method (12). Purity was about 75% on Gas Liquid Chromatograph analysis. (Gas Liquid Chromatograph was done on Aerograph HY-FI model 600-D. H_2 flow rate at 25.5 ml/min, range at 10, temperature at 287°C.)

homogenized in a test tube with a close-fitting power-driven teflon pestol for two minutes. The homogenization was interrupted after one minute to permit rechilling of the suspension. A 10 ml aliquot of the homogenate was layered carefully over 10 ml of 0.34 M sucrose and the mixture was centrifuged for 10 minutes at 2,000 r.p.m. The centrifuge was accelerated and decelerated slowly to avoid mixing of the two layers. The supernatant was centrifuged for 10 minutes at 9,200 r.p.m. The opalescent supernatant and the pink partially sedimented layer of particles above the firmly packed pellet of mitochondria were removed. The pellet was transferred quantitatively with 0.25 M sucrose and was redispersed by homogenization in 5 ml of the sucrose solution and was centrifuged at 20,000 r.p.m. for 10 minutes. This procedure was repeated. The final suspension of thrice-sedimented mitochondria was used as the enzyme preparation. In all cases the mitochondrial suspensions were assayed for nitrogen content by Koch's colorimetric method (29).

The oxygen required during incubation of oleic acid with the mitochondria from the liver of the rats fed the safflower oil diet and CPFA diet both served as the controls. The oxygen consumption was compared between two substrates, methyl sterculate and oleic acid, in the liver mitochondria of safflower oil and CPFA fed rats. The cross comparison was made to show any difference in catabolic function based on oxygen consumption between safflower oil fed rats and cyclopropene fatty acid fed rats (Table 4).

Table 4. Cross Comparison Design

Liver Mitochondria	Substrate	
	Oleic Acid	Methyl Sterculate
4% safflower oil diet	$4x10^{-4}$ M	$4x10^{-4}$ M
1% safflower oil +3% *Sterculia foetida* oil diet	$4x10^{-4}$ M	$4x10^{-4}$ M

The Reaction Medium

The reagents were the same as suggested by Lehninger (30). The incubations were conducted in 15 ml Warburg flasks. In each flask, 0.2 ml of twenty per cent KOH was put in the center wall, a 2x2 cm^2 filter paper wick was suspended in the KOH solution for CO_2 collection. The incubation medium contained ATP[3] (7.5 µM), cytochrome C[4] (0.045 µM), $MgCl_2$ (15 µM), KCl (100 µM), sodium-potassium phosphate buffer (40 µM, PH 7.4), mitochondria in 0.25 M sucrose (equivalent to 0.1125 g of fresh liver), fatty acid-albumin[5] complex (6 µM of fatty acid per 70 mgs albumin[6]). The reaction flasks were equilibrated in the Warburg

3. ATP; disodium salt was purchased at P-L Chemicals, Inc., Milwaukee, Wisconsin.

4. Cytochrome C; from horse heart, type 3, was purchased at Sigma Chemical Co., St. Louis, Missouri.

5. Fatty acid; oleic acid was purchased from Nutritional Biochemicals Co., Cleveland, Ohio. The oleic acid content was about 80% on Gas Liquid Chromatograph analysis. (Gas Liquid Chromatography was done on Aerograph HY-FI model 600-D. H_2 flow rate at 25.5 ml/min, range at 10, temperature at 287°C.)

6. Albumin; bovine albumin fraction 5 was purchased from Sigma Chemical Co., St. Louis, Missouri.

water bath at 30°C for 10 minutes. Four readings were taken at successive 10 minute intervals followed by two readings at 20 minute intervals for a total period of 80 minutes. The control flasks had all the reagents but no substrate during the reaction period. It was used as the thermo barometer. The reactions were stopped by adding twenty-five per cent trichloroacetic acid to a final concentration of three per cent. The trichloroacetic acid precipitates were collected at 0, 20, 40, and 80 minutes (Table 5).

The Nitrogen Determination

Mitochondria were assayed for nitrogen content by Koch's colorometric method (29). To 0.5 ml of mitochondria suspension, 1 cc of 18N sulfuric acid solution was added and three beads were placed in the test tube to prevent bumping. These were heated over a microburner in a hood until dense white fumes filled the tube. At this point, the tube was removed from the flame and cooled for thirty seconds to one minute. Then, five to ten drops of thirty per cent hydrogen peroxide were added. After the bubbling stopped, these were again heated to boiling for five minutes, cooled to room temperature and diluted to thirty-five ml with water. Tubes were placed in an ice water bath until the temperature dropped to 11°C, 15 ml of Nessler's solution[7] was added to each tube, read immediately in a colorimeter at 520 mμ and compared to a standard curve.

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7. Nessler's solution was purchased from Fisher Scientific Co., Fair Lawn, New Jersey.

Table 5. Composition of Reaction Medium

Reaction Medium				
Reagents	Center Wall	Main Compartment	Concentration	Dilute Agent
ATP	---	0.5 ml	1.5×10^{-2} M	H_2O*
Cytochrome C	---	0.15 ml	3×10^{-4} M	Na-K-buffer
$MgCl_2$	---	0.15 ml	1×10^{-1} M	H_2O
KCl	---	0.1 ml	1 M	H_2O
Na-K-buffer	---	0.4 ml	PH = 7.4	---
H_2O	---	0.16 ml	---	---
Fatty acid	---	0.04 ml	1×10^{-2} M	H_2O, albumin
Mitochondria	---	0.5 ml	---	---
KOH	0.2 ml	---	20%	H_2O
Filter paper	2x2 cm^2	---	---	---

*Deionized water.

Lipid Extraction

The trichloroacetic acid precipitates were extracted with chloroform-methanol (31) and were esterified with 0.5 N $NaOCH_3$ and 7% BF_3 in CH_3OH. The resulting fatty esters were analyzed using an Aerograph HY-FI model 600-D gas chromatograph equipped with a hydrogen flame detector. The column used was 5 ft. x 1/8 in. stainless steel packed with acid-washed chromosorb W 80/100 mesh and coated with twenty per cent diethyleneglycol succinate. Column temperature was held at 185°C and the flow rate of hydrogen was maintained at 25.5 to 26 mls/min.

RESULTS AND DISCUSSIONS

The optimum amount of methyl sterculate substrate for mannometric respiration measurement was studied at concentrations of $2x10^{-4}$ M, $4x10^{-4}$ M, and $6x10^{-4}$ M (Fig. 3). The oxidation of methyl sterculate by liver mitochondria from the safflower oil diet fed rats or the CPFA fed rats was proportional to the concentrations of the methyl sterculate after eighty minutes incubation. The oxygen consumption of the safflower oil fed group was about 40 µl/mg N_2 more than the CPFA fed group at each of the three substrate concentrations. In this experiment a concentration of $4x10^{-4}$ M was chosen for the mannometric respiration studies.

Sheehan (1967) reported that the rats fed Sterculia foetida oil had reduced body weight and increased liver weight. The increased weight of the livers was accompanied by an increased content of saturated fatty acids (4). It is possible that the fatty acyl desaturase enzyme system was blocked during the feeding of methyl sterculate. The increased saturated fatty acids might cause some permanent cytotoxic effect on liver mitochondria. Thus, the oxidation of oleic acid in the liver mitochondria of CPFA fed rats was less effective than the safflower oil diet fed rats.

Anderson (1967) demonstrated that the rat liver mitochondria are able to convert all of the carbon atoms of both the cis and trans isomers of Δ9-octadecenoic acid to CO_2, acetoacetic acid and

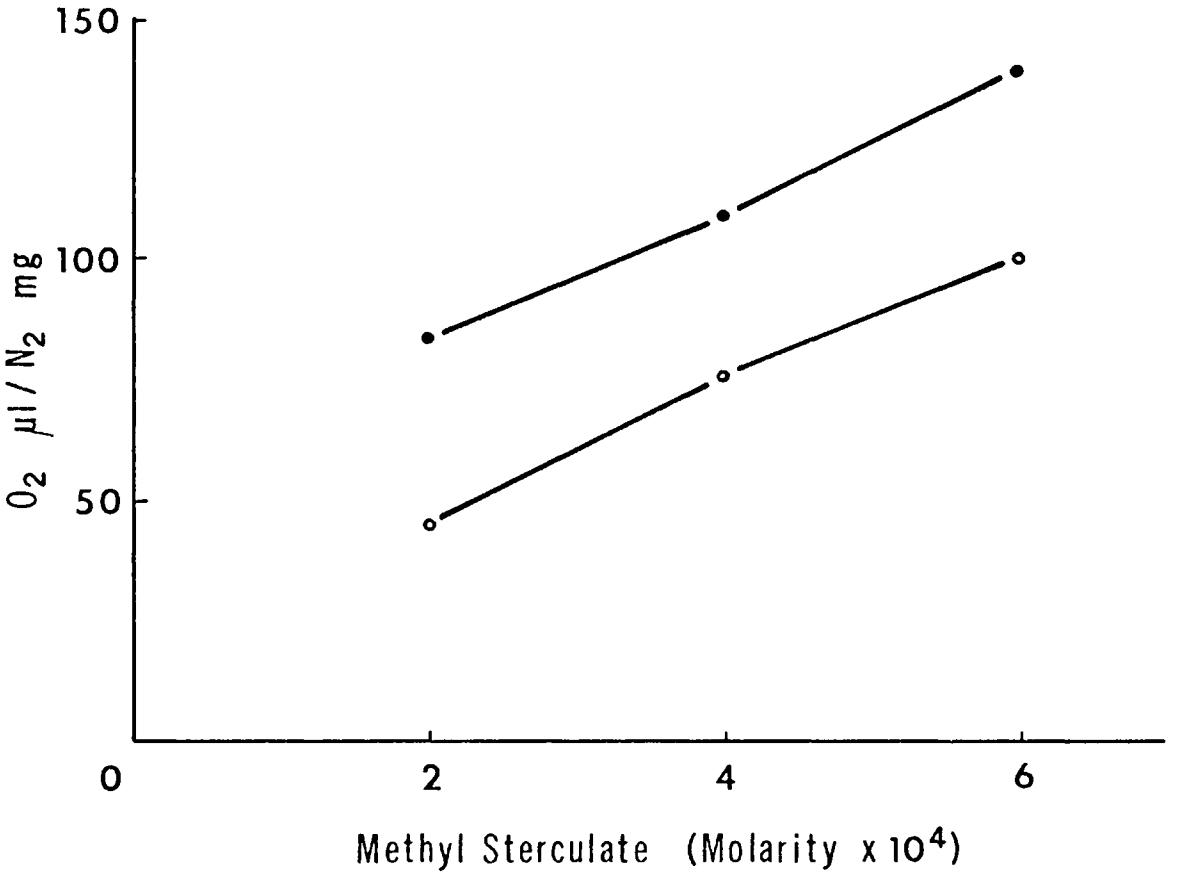

Fig. 3. Oxygen Consumption of Liver Mitochondria of Safflower Oil Fed Rats and CPFA Fed Rats in Different Concentrations of Methyl Sterculate in 80 minutes.

● - rats fed 4% safflower oil diet
o - rats fed 1% safflower oil and 3% sterculia foetida oil diet

beta-hydroxybutyric acid, thus, beta-oxidation was the major route of monoenoic acid oxidation in liver mitochondria (32,33,34).

During the reaction of liver mitochondria from safflower oil fed rats with $4x10^{-4}$ M oleic acid as the substrate, the oxygen consumption was directly proportional to the reaction time. When methyl sterculate was used as the substrate, the oxygen consumption also was directly proportional to the reaction time, but at a higher consumption rate. The amount of oxygen consumption per period of time in methyl sterculate was more than twice that of oleic acid (Fig. 4).

When liver mitochondria from CPFA fed rats metabolized oleic acid as the substrate, the oxygen consumption was proportional to the reaction time, but when methyl sterculate was the substrate, a decrease in oxygen consumption was observed after 40 minutes reaction time (Fig. 5).

The results (Tables 6 and 7), when oleic acid was used as the substrate in the media of the liver mitochondria of the safflower oil fed rats, the consumption of oxygen (O_2 µl/mg N_2) was 13.8, 25.6, and 46.6 after 20, 40, and 80 minutes incubation, respectively. Changing the substrate of oleic acid to methyl sterculate, after the same periods of incubation, the oxygen consumptions increased to 32.1, 63.9, and 110.6, respectively. In the liver mitochondria of the CPFA fed rats, the oxygen consumption was 11.4, 23.4, and 44.1 when oleic acid was used, or 29.3, 53.6, and 75.9 when methyl sterculate was used as the substrate. These results showed that with methyl sterculate as the

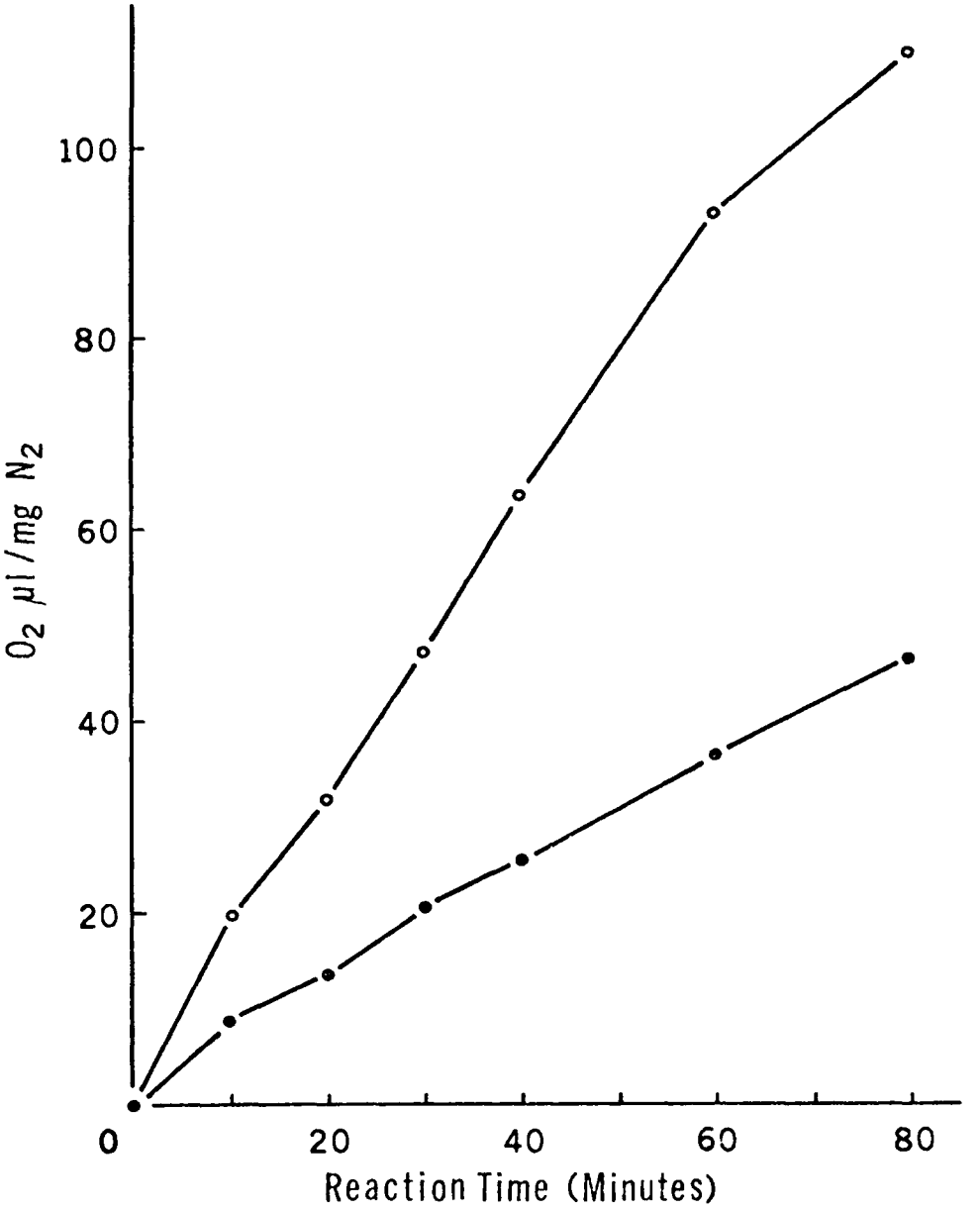

Fig. 4. Oxygen Consumption of Liver Mitochondria of Safflower Oil Fed Rats with Oleic Acid or Methyl Sterculate as Substrate at $4x10^{-4}$ M Concentration.

● - $4x10^{-4}$ M of oleic acid
o - $4x10^{-4}$ M of methyl sterculate

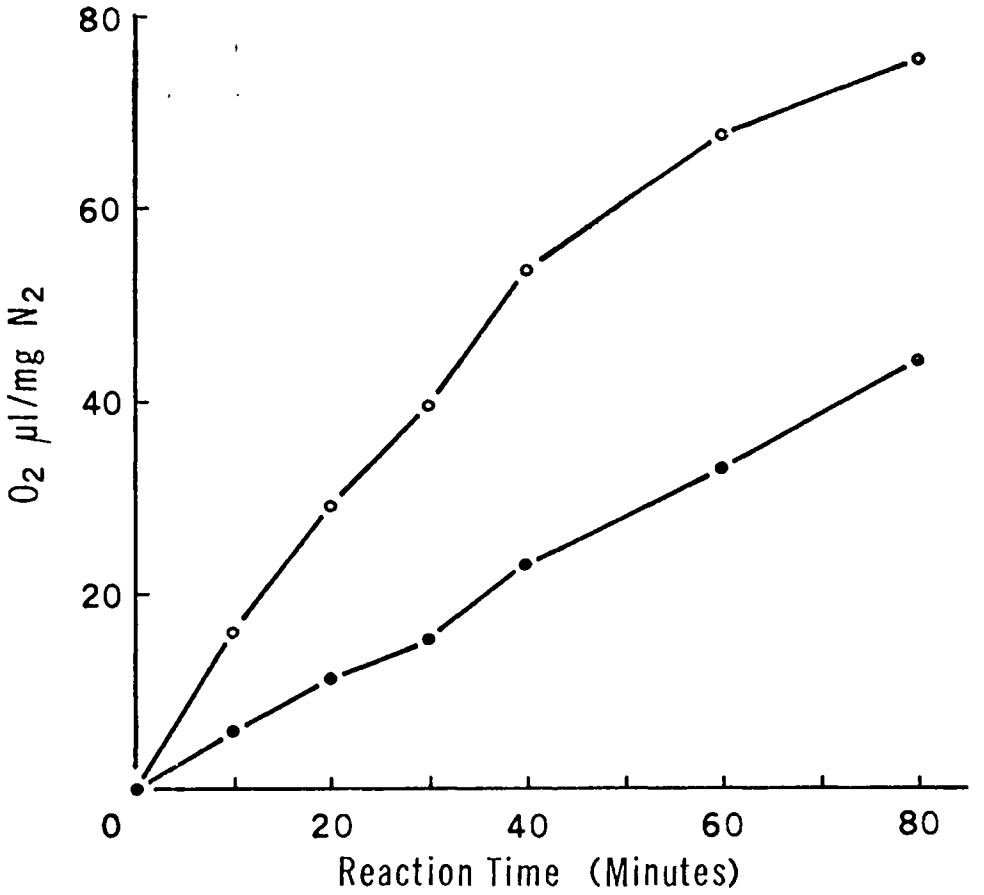

Fig. 5. Oxygen Consumption of Liver Mitochondria of CPFA Fed Rats with Oleic Acid or Methyl Sterculate as the Substrate at $4x10^{-4}$ M Concentration.

● - $4x10^{-4}$ M oleic acid
o - $4x10^{-4}$ M methyl sterculate

Table 6. Comparison of Stearic Acid and Oleic Acid Ratios and Respiration Rates in Liver Mitochondria of Safflower Oil Fed Rats and CPFA Fed Rats with Oleic Acid as Substrate

Time in Minutes	Liver Mitochondria from Safflower Oil Fed Rats				Liver Mitochondria from CPFA Fed Rats			
	Stearic Acid	Oleic Acid	Respiration	Stearic Acid / Oleic Acid	Stearic Acid	Oleic Acid	Respiration	Stearic Acid / Oleic Acid
	%	%	O_2 µl/mg N_2		%	%	O_2 µl/mg N_2	
0	46.1*	53.9*	0	0.85	58.5*	41.5*	0	1.41
20	51.0	49.0	13.8	1.04	59.0	41.0	11.4	1.44
40	51.9	68.1	25.6	1.08	61.6	38.4	23.4	1.60
80	52.5	47.5	46.6	1.11	62.8	37.2	44.1	1.69

*The total amount of stearic acid and oleic acid is computed as 100%.

Table 7. Comparison of Stearic Acid and Oleic Acid Ratios and Respiration Rates in Liver Mitochondria of Safflower Oil Fed and CPFA Fed Rats with Methyl Sterculate as Substrate

Time in Minutes	Liver Mitochondria from Safflower Oil Fed Rats				Liver Mitochondria from CPFA Fed Rats			
	Stearic Acid	Oleic Acid	Respiration	Stearic Acid / Oleic Acid	Stearic Acid	Oleic Acid	Respiration	Stearic Acid / Oleic Acid
	%	%	O_2 µl/mg N_2		%	%	O_2 µl/mg N_2	
0	53.0*	47.0*	0	1.13	62.2*	37.8	0	1.64
20	53.5	46.5	32.1	1.15	63.5	36.5	29.3	1.74
40	54.6	45.4	63.9	1.20	63.5	36.5	53.6	1.74
80	55.1	44.9	110.6	1.23	63.8	36.2	75.9	1.76

*The total amount of stearic acid and oleic acid is computed as 100%.

substrate, two to two and one half times more oxygen was consumed than when oleic acid was used as substrate.

The oxidation of fatty acids to CO_2 and H_2O occurs by the sequential combination of two multienzyme systems; first, the beta-oxidation cycle converts fatty acid to acetoacetic acid and H_2O, and the tricarboxylic acid cycle converts the acetoacetic acid to CO_2 and H_2O. The oxygen needed for oxidation of fatty acid through the first cycle is much less than if it proceeds farther via tricarboxylic acid cycle to give CO_2 and H_2O as end products.

Lehninger (1955) suggested that rat liver mitochondria preparations were the choice for studying the sequence of fatty acid to acetoacetic acid and H_2O. The rat kidney mitochondria preparation was the choice for studying the sequence of fatty acid to CO_2 and H_2O.

Theoretically, if beta-oxidation could not pass the cyclopropene ring, the oxygen consumption of cyclopropene fatty acid should be less than that of oleic acid during beta-oxidation process. On the other hand, if beta-oxidation could proceed and pass the cyclopropene ring, due to the similar carbon content in these fatty acids, the oxygen consumption should be approximately equal.

The greater amount of oxygen consumption in methyl sterculate than in oleic acid was contradictory to the above theory. At the present time it is very difficult to use the limited knowledge of fatty acid oxidation to try to explain this observation.

The metabolic reactions of the media containing the liver mitochondria from safflower oil fed rats with added oleic acid or

methyl sterculate as substrate were stopped by adding trichloroacetic acid at 0, 20, 40, and 80 minutes. Then lipid extractions were made from these media and analyzed by gas liquid chromatography. The concentrations of stearic acid and the oleic acid were compared and the total amount of stearic acid plus oleic acid was computed as 100 per cent. An increase in stearic/oleic acid ratio during the incubation time indicates the decrease of oleic acid or its oxidation.

In the liver mitochondria of safflower oil fed rats, with oleic acid as the substrate at 0 time, the ratio of stearic acid to oleic acid was 0.85. After 80 minutes reaction period, the ratio became 1.11. The stearic acid was increased thirty per cent, whereas in the CPFA fed rats' liver mitochondria the ratio of stearic acid to oleic acid during 80 minutes reaction period was increased from 1.41 to 1.69. This represented an increase of about twenty per cent (Table 6).

When methyl sterculate was used as the substrate, the liver mitochondria of the safflower oil fed rats oxidized the oleic acid slightly better than the mitochondria of the liver of the CPFA fed group (Table 7). In the prior case the ratio of stearic acid to oleic acid was 1.13 to 1. It became 1.23 to 1 at the end of 80 minutes reaction period. The increase was nine per cent. In the latter case the ratio change was from 1.64 to 1.76. There was only seven per cent increase in the ratio of stearic acid and oleic acid.

In the cross examination of the above data, the liver mitochondria from safflower oil fed rats oxidized the oleic acid about thirty per cent during an 80 minute period when oleic acid was used as

the substrate. When methyl sterculate served as the substrate in the media, the relative oxidation of oleic acid was reduced to nine per cent. In the liver mitochondria from CPFA fed rats, a change of the substrate from oleic acid to methyl sterculate resulted in a reduction of the relative oxidation of oleic acid from twenty per cent to seven per cent. These results indicated that the methyl sterculate, indeed, interfered with the normal fatty acid oxidation process.

The difference in respiration indicated that the metabolic mechanism of methyl sterculate in rat liver mitochondria was different from the same carbon-chain length mono-unsaturated fatty acid. Further investigation is needed to find out why it consumed more oxygen.

CONCLUSIONS

The evaluation of fatty acid catabolism via beta-oxidation cycle in liver mitochondria from safflower oil and CPFA fed rats, using methyl sterculate and oleic acid as substrates showed following results:

1. More than two times as much oxygen was consumed in the media with substrate of methyl sterculate than with oleic acid.

2. The ratio of stearic acid to oleic acid was higher in the media contained liver mitochondria from CPFA fed rats than from safflower oil fed rats.

3. The increase in the ratio of stearic acid to oleic acid was greater in both media with substrate of oleic acid than with methyl sterculate during 80 minutes incubation period.

The reason for the greater oxygen consumption with methyl sterculate as the substrate was not apparent.

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